(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 July 2003 (17.07.2003)

PCT

(10) International Publication Number
WO 03/057200 A2

(51) International Patent Classification[7]: A61K 31/00

(21) International Application Number: PCT/DK03/00017

(22) International Filing Date: 13 January 2003 (13.01.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PA 2002 00047 11 January 2002 (11.01.2002) DK
60/348,332 14 January 2002 (14.01.2002) US

(71) Applicant (*for all designated States except US*): NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK).

(72) Inventor; and
(75) Inventor/Applicant (*for US only*): CARR, Richard, David [GB/DK]; Munkevej 27, DK-3500 Værløse (DK).

(74) Common Representative: NOVO NORDISK A/S; Corporate Patents, Novo Allé, DK-2880 Bagsværd (DK).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/057200 A2

(54) Title: METHOD AND COMPOSITION FOR TREATMENT OF DIABETES, HYPERTENSION, CHRONIC HEART FAILURE AND FLUID RETENTIVE STATES

(57) Abstract: The present invention is related to a method and composition for treatment of diabetes, hy-pertension, chronic heart failure and fluid retentive states comprising administering inhibitors of the enzymes NEP and DPP-IV to individuals suffering from one or more of those condi-tions. Inhibition of the activity of the two enzymes will potentiate the insulin releasing activity of endogenous GLP-1 and other DPP-IV substrates like GIP

TITLE

Method and composition for treatment of diabetes, hypertension, chronic heart failure and fluid retentive states

FIELD OF THE INVENTION

This invention relates to a pharmaceutical composition comprising a Dipeptidyl Peptidase-IV inhibitor in combination with an inhibitor of Neutral Endopeptidase.

BACKGROUND OF THE INVENTION

Dipeptidyl peptidase-IV (DPP-IV), a serine protease belonging to the group of post-proline/alanine cleaving amino-dipeptidases, specifically removes the two N-terminal amino acids from proteins having proline or alanine in position 2.

Although the physiological role of DPP-IV has not been completely established, it is believed to play an important role in neuropeptide metabolism, T-cell activation, gastric ulceration, functional dyspepsia, obesity, appetite regulation, impaired fasting glucose (IFG) and diabetes.

DPP-IV has been implicated in the control of glucose metabolism because its substrates include the insulinotropic hormones Glucagon like peptide-1 (GLP-1) and Gastric inhibitory peptide (GIP). GLP-1 and GIP are active only in their intact forms; removal of their two N-terminal amino acids inactivates them. It is also speculated that other, as yet unknown substrates may participate in the beneficial effects of DPP-IV inhibitors in treatment of diabetes

In vivo administration of synthetic inhibitors of DPP-IV prevents N-terminal degradation of GLP-1 and GIP, resulting in higher plasma concentrations of these hormones, increased insulin secretion and, therefore, improved glucose tolerance. Therefore, such inhibitors have been proposed for the treatment of patients with Type 2 diabetes, a disease characterised by decreased glucose tolerance. (Holst, J. J.; Deacon, C. F. Diabetes 47 (1998) 1663-70)

Diabetic dyslipidaemia is characterized by multiple lipoprotein defects, including moderately high serum levels of cholesterol and triglycerides, small LDL particles, and low levels of HDL cholesterol. The results of recent clinical trials reveal beneficial effects of cholesterol-lowering therapy in diabetic and non-diabetic patients, thus supporting increased emphasis on treatment of diabetic dyslipidaemia. The National Cholesterol Education Program's Adult Treatment Panel II advocated this need for intensive treatment of diabetic dyslipidaemia.

Obesity is a well-known risk factor for the development of many very common diseases such as atherosclerosis, hypertension and diabetes. The incidence of obese people and thereby also these diseases is increasing throughout the entire industrialised world. Except for exer-

cise, diet and food restriction no convincing pharmacological treatment for reducing body weight effectively and acceptably currently exist. However, due to its indirect but important effect as a risk factor in mortal and common diseases it will be important to find treatment for obesity or appetite regulation. Even mild obesity increases the risk for premature death, diabetes, hypertension, atherosclerosis, gallbladder disease and certain types of cancer. In the industrialised western world the prevalence of obesity has increased significantly in the past few decades. Because of the high prevalence of obesity and its health consequences, its prevention and treatment should be a high public health priority.

At present a variety of techniques are available to effect initial weight loss. Unfortunately, initial weight loss is not an optimal therapeutic goal. Rather, the problem is that most obese patients eventually regain their weight. An effective means to establish and/or sustain weight loss is the major challenge in the treatment of obesity today.

Neutral Endopeptidase (NEP) is an enzyme known to be responsible for the metabolism of polypeptide hormones (e.g. atrial natriuretic factor and brain (B-type) natriuretic factor) which are involved with the regulation of extracellular fluid (volume/water/sodium ion) homeostasis. Furthermore, NEP is known to be involved in the metabolism of other biologically active peptides.

NEP inhibitors are useful as they are diuretic agents and, as such, are known medicines in the treatment if hypertension and chronic heart failure. They are effective at reducing peripheral vascular resistance and lowering the circulating volume (thus lowering cardiac preload). They are useful when treating both cardiac and non-cardiac sources of oedema. Hypertension and chronic heart failure are life threatening diseases, which increase the risk of cerebrovascular stroke and myocardial infarction. Diuretic agents, including thiazide diuretics and loop diuretics provide important drug therapy for these two disorders. NEP inhibitors, either administered alone (Westheim, AS., Bostrom, P., Christensen, CC.et al J Am Coll Cardiol., 1999, 34: 1794-1801), or in combination with ACE inhibitors (Newby, DE., McDonagh, T., Currie, PF. et al Eur Heart J., 1998; 19: 1808-1813) have also been shown to cause favourable effects in these disease states. Furthermore compounds showing dual inhibition of both NEP and ACE (e.g. omapatrilat) show promise in treatment of hypertension and heart failure (Trippodo, NC., Fox, M,. Monticello, TM et al J Cardiovasc. Pharmacol., 1999; 34: 782-790) as a result of the useful addition of the two effects of the component drugs on polypeptide levels. NEP is also reported to be involved in metabolism of GLP-1 (Hupe-Sodmann, K., McGregor, GP., Bridenbaugh, R et al Regulatory Peptides 1995; 58: 149-56, Hupe-Sodmann, K, Goeke, R., Goeke, B et al Peptides 1997; 18: 625-32).

DEFINITIONS

The term "DPP-IV" as used herein is intended to mean Dipeptidyl peptidase IV (EC 3.4.14.5; DPP-IV), also known as CD26. DPP-IV cleaves a dipeptide from the N terminus of a polypeptide chain containing a proline or alanine residue in the penultimate position.

The term "NEP" as used herein is intended to mean Neutral Endopeptidase (E.C. 3.4.24.11; NEP).

The term "ACE" as used herein is intended to mean Angiotensin Converting Enzyme (E.C. 3.4.15.1; ACE).

The term "inhibitor" is intended to indicate a molecule that exhibits inhibition of the enzymatic activity of the indicated enzyme, such as from 1-100% inhibition. The enzymatic activity of DPP-IV may be measured in the assay as described in the section "Methods for measuring the activity of compounds which inhibit the enzymatic activity of CD26/DPP-IV".

In the present context "an inhibitor" is also intended to comprise active metabolites and prodrugs thereof, such as active metabolites and prodrugs of the inhibitors. A "metabolite" is an active derivative of an inhibitor produced when the inhibitor is metabolised. A "prodrug" is a compound that is either metabolised to an inhibitor or is metabolised to the same metabolite(s) as an inhibitor.

The term "hypoglycaemia" is a condition of low blood sugar levels, for example a blood sugar level below 4 mmol/l, such as below 3 mmol/l, for example below 2.5 mmol/l, such as below 2 mmol/l.

By the term "treatment" is understood the management and care of a patient for the purpose of combating the disease, condition, or disorder.

The term "beta cell degeneration" is intended to mean loss of beta cell function, beta cell dysfunction, and death of beta cells, such as necrosis or apoptosis of beta cells.

The term "Impaired Glucose Tolerance" (IGT) is intended to mean a condition indicated by a 2-h postload glucose (2-h PG) between 7.8 mmol/l and 11.1 mmol/l in an Oral Glucose Tolerance Test (OGTT) using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

The term "Impaired Fasting Glucose" (IFG) is intended to mean a condition indicated by a Fasting Plasma Glucose (FPG) between 6.1 mmol/l and 7.0 mmol/l, where fasting is defined as no caloric intake for at least 8 hours.

The term "non-insulin demanding type 2 diabetes" is intended to mean a condition where the individual has insulin resistance, insulin deficiency and either a FPG of more than 7.0 mmol/l or a 2-h PG of more than 11.1 mmol/l when untreated, and where normoglycemia can be achieved without insulin injections.

The term "insulin-demanding type 2 diabetes" is intended to mean a condition where the individual has insulin resistance, insulin deficiency and either a FPG of more than 7.0 mmol/l or a 2-h PG of more than 11.1 mmol/l when untreated, and where normoglycemia can only be achieved with insulin injections.

The term "C_1-C_{10} alkyl" as used herein, alone or in combination, refers to a straight or branched, saturated hydrocarbon chain having from 1-10 carbon atoms such as but not limited to e.g. methyl, ethyl, n-propyl, isopropyl, n-butyl, sec. Butyl, isobutyl, tert. Butyl, n-pentyl, 2-methylbutyl, 3-methylbutyl, n-hexyl, 4-methylpentyl, neopentyl, 2,2-dimethylpropyl and the like.

The term "C_2-C_{10}-alkenyl" used herein, alone or in combination, refers to a straight or branched, unsaturated hydrocarbon chain having from 2-10 carbon atoms and at least one double bond such as but not limited to vinyl, 1-propenyl, allyl, isopropenyl, n-butenyl, n-pentenyl and n-hexenyl and the like.

The term "C_2-C_{10} alkynyl" as used herein, alone or in combination, refers to an unsaturated hydrocarbon chain having from 2-10 carbon atoms and at least one triple bond such as but not limited to $-C{\equiv}CH$, $-C{\equiv}CCH_3$, $-CH_2C{\equiv}CH$, $-CH_2$-CH_2-$C{\equiv}CH$, $-CH(CH_3)\,C{\equiv}CH$ and the like.

The term "$C_{1\text{-}10}$-alkoxy" as used herein, alone or in combination is intended to include those $C_{1\text{-}10}$-alkyl groups of the designated length in either a linear or branched or cyclic configuration linked through an ether oxygen having its free valence bond from the ether oxygen. Examples of linear alkoxy groups are methoxy, ethoxy, propoxy, butoxy, pentoxy and hexoxy. Examples of branched alkoxy are isopropoxy, sec-butoxy, tert-butoxy, isopentoxy and isohexoxy. Example of cyclic alkoxy are cyclopropyloxy, cyclobutyloxy, cyclopentyloxy and cyclohexyloxy.

The term "C_3-C_{10} cycloalkyl" as used herein refers to a radical of one or more saturated cyclic hydrocarbon having from 3-10 carbon atoms such as but not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, adamantyl and the like.

The term "C_5-C_{10} cycloalkenyl" as used herein refers to a radical of one or more cyclic hydrocarbon having at least one double bond having from 5-10 carbon atoms such as but not limited to cyclopentenyl, cyclohexenyl and the like

The term "C_2-C_6 cycloheteroalkyl" as used herein refers to a radical of totally saturated heterocycle like a cyclic hydrocarbon containing one or more heteroatoms selected from nitrogen, oxygen and sulphur independently in the cycle such as pyrrolidine (1- pyrrolidine; 2- pyr-

rolidine; 3- pyrrolidine; 4- pyrrolidine; 5- pyrrolidine); pyrazolidine (1- pyrazolidine; 2- pyrazolidine; 3- pyrazolidine; 4-pyrazolidine; 5-pyrazolidine); imidazolidine (1- imidazolidine; 2-imidazolidine; 3- imidazolidine; 4- imidazolidine; 5- imidazolidine); thiazolidine (2- thiazolidine; 3- thiazolidine; 4- thiazolidine; 5- thiazolidine); piperidine (1- piperidine; 2-piperidine; 3- piperidine; 4- piperidine; 5- piperidine; 6- piperidine); piperazine (1- piperazine; 2- piperazine; 3- piperazine; 4- piperazine; 5- piperazine; 6- piperazine); morpholine (2- morpholine; 3- morpholine; 4- morpholine; 5- morpholine; 6- morpholine); thiomorpholine (2-thiomorpholine; 3- thiomorpholine; 4- thiomorpholine; 5- thiomorpholine; 6- thiomorpholine); 1,2-oxathiolane (3-(1,2-oxathiolane); 4-(1,2-oxathiolane); 5-(1,2-oxathiolane); 1,3-dioxolane (2-(1,3-dioxolane); 4-(1,3-dioxolane); 5-(1,3-dioxolane); tetrahydropyrane; (2-tetrahydropyrane; 3-tetrahydropyrane; 4-tetrahydropyrane; 5-tetrahydropyrane; 6-tetrahydropyrane); hexahydropyridazine (1-(hexahydropyridazine); 2-(hexahydropyridazine); 3-(hexahydropyridazine); 4-(hexahydropyridazine); 5-(hexahydropyridazine); 6-(hexahydropyridazine)).

The term "aryl" as used herein includes carbocyclic aromatic ring systems. Aryl is also intended to include the partially hydrogenated derivatives of the carbocyclic systems.

The term "heteroaryl" as used herein includes heterocyclic unsaturated ring systems containing one or more heteroatoms selected from nitrogen, oxygen and sulphur such as furyl, thienyl, pyrrolyl, heteroaryl is also intended to include the partially hydrogenated derivatives of the heterocyclic systems enumerated below.

The terms "aryl" and "heteroaryl" as used herein refers to an aryl which can be optionally substituted or a heteroaryl which can be optionally substituted and includes phenyl, biphenyl, indenyl, naphthyl (1-naphthyl, 2-naphthyl), N-hydroxytetrazolyl, N-hydroxytriazolyl, N-hydroxyimidazolyl, anthracenyl (1-anthracenyl, 2-anthracenyl, 3-anthracenyl), thiophenyl (2-thienyl, 3-thienyl), furyl (2-furyl, 3-furyl), indolyl, oxadiazolyl, isoxazolyl, quinazolinyl, fluorenyl, xanthenyl, isoindanyl, benzhydryl, acridinyl, thiazolyl, pyrrolyl (2-pyrrolyl), pyrazolyl (3-pyrazolyl), imidazolyl (1-imidazolyl, 2-imidazolyl, 4-imidazolyl, 5-imidazolyl), triazolyl (1,2,3-triazol-1-yl, 1,2,3-triazol-2-yl 1,2,3-triazol-4-yl, 1,2,4-triazol-3-yl), oxazolyl (2-oxazolyl, 4-oxazolyl, 5-oxazolyl), thiazolyl (2-thiazolyl, 4-thiazolyl, 5-thiazolyl), pyridyl (2-pyridyl, 3-pyridyl, 4-pyridyl), pyrimidinyl (2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, 6-pyrimidinyl), pyrazinyl, pyridazinyl (3- pyridazinyl, 4-pyridazinyl, 5-pyridazinyl), quinolyl (2-quinolyl, 3-quinolyl, 4-quinolyl, 5-quinolyl, 6-quinolyl, 7-quinolyl, 8-quinolyl), isoquinolyl (1-isoquinolyl, 3-isoquinolyl, 4-isoquinolyl, 5-isoquinolyl, 6-isoquinolyl, 7-isoquinolyl, 8-isoquinolyl), benzo[b]furanyl (2-benzo[b]furanyl, 3-benzo[b]furanyl, 4-benzo[b]furanyl, 5-benzo[b]furanyl, 6-benzo[b]furanyl, 7-benzo[b]furanyl), 2,3-dihydro-benzo[b]furanyl (2-(2,3-dihydro-benzo[b]furanyl), 3-(2,3-

dihydro-benzo[b]furanyl), 4-(2,3-dihydro-benzo[b]furanyl), 5-(2,3-dihydro-benzo[b]furanyl), 6-(2,3-dihydro-benzo[b]furanyl), 7-(2,3-dihydro-benzo[b]furanyl), benzo[b]thiophenyl (2-benzo[b]thiophenyl, 3-benzo[b]thiophenyl, 4-benzo[b]thiophenyl, 5-benzo[b]thiophenyl, 6-benzo[b]thiophenyl, 7-benzo[b]thiophenyl), 2,3-dihydro-benzo[b]thiophenyl (2-(2,3-dihydro-benzo[b]thiophenyl), 3-(2,3-dihydro-benzo[b]thiophenyl), 4-(2,3-dihydro-benzo[b]thiophenyl), 5-(2,3-dihydro-benzo[b]thiophenyl), 6-(2,3-dihydro-benzo[b]thiophenyl), 7-(2,3-dihydro-benzo[b]thiophenyl), indolyl (1-indolyl, 2-indolyl, 3-indolyl, 4-indolyl, 5-indolyl, 6-indolyl, 7-indolyl), indazole (1-indazolyl, 3-indazolyl, 4-indazolyl, 5-indazolyl, 6-indazolyl, 7-indazolyl), benzimidazolyl (1-benzimidazolyl, 2-benzimidazolyl, 4-benzimidazolyl, 5-benzimidazolyl, 6-benzimidazolyl, 7-benzimidazolyl, 8-benzimidazolyl), benzoxazolyl (1-benzoxazolyl, 2-benzoxazolyl), benzothiazolyl (1-benzothiazolyl, 2-benzothiazolyl, 4-benzothiazolyl, 5-benzothiazolyl, 6-benzothiazolyl, 7-benzothiazolyl), carbazolyl (1-carbazolyl, 2-carbazolyl, 3-carbazolyl, 4-carbazolyl), 5H-dibenz[b,f]azepine (5H-dibenz[b,f]azepin-1-yl, 5H-dibenz[b,f]azepine-2-yl, 5H-dibenz[b,f]azepine-3-yl, 5H-dibenz[b,f]azepine-4-yl, 5H-dibenz[b,f]azepine-5-yl), 10,11-dihydro-5H-dibenz[b,f]azepine (10,11-dihydro-5H-dibenz[b,f]azepine-1-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-2-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-3-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-4-yl, 10,11-dihydro-5H-dibenz[b,f]azepine-5-yl).

The term halogen as used herein refers to fluorine, chlorine, bromine or iodine.
The term "antidiabetic" is meant to encompass any substance or pharmaceutical composition, which can be used for prophylactic, ameliorative or curative treatment of diabetes mellitus, wherein diabetes mellitus may be any type of diabetes.

Suitable antidiabetics comprise insulin, GLP-1 derivatives such as those disclosed in WO 98/08871 (Novo Nordisk A/S), which is incorporated herein by reference as well as orally active hypoglycemic agents.

In one preferred embodiment the antidiabetic is insulin or an analogue thereof or a derivative thereof. More preferably the antidiabetic is human insulin or an analogue thereof or a derivative thereof. However, porcine insulin is also an insulin species, which may be employed with the present invention. Preferably, porcine insulin is highly purified naturally produced porcine insulin.

Human insulin could be naturally produced insulin, preferably however human insulin is recombinantly produced. Recombinant human insulin may be produced in any suitable host cell for example the host cells may be bacterial, fungal (including yeast), insect, animal or plant cells. Preferably, the host cells are yeast cells or bacterial cells such as for example *E. coli*.

Preferably, the analogue of human insulin is a rapid-acting analogue. For example the analogue may be selected from the group consisting of AspB28 human insulin and LysB28ProB29 human insulin.

In one preferred embodiment the derivative is human insulin or an analogue thereof containing a C_8 to C_{40} lipophilic substituent in position B29. Preferably, the derivative may be selected from the group consisting of B29-N^{ε}-myristoyl-des(B30) human insulin, B29-N^{ε}-palmitoyl-des(B30) human insulin, B29-N^{ε}-myristoyl human insulin, B29-N^{ε}-palmitoyl human insulin, B28-N^{ε}-myristoyl Lys^{B28} Pro^{B29} human insulin, B28-N^{ε}-palmitoyl Lys^{B28} Pro^{B29} human insulin, B30-N^{ε}-myristoyl-$Thr^{B29}Lys^{B30}$ human insulin, B30-N^{ε}-palmitoyl-$Thr^{B29}Lys^{B30}$ human insulin, B29-N^{ε}-(N-palmitoyl-γ-glutamyl)-des(B30) human insulin, B29-N^{ε}-(N-lithocholyl-γ-glutamyl)-des(B30) human insulin, B29-N^{ε}-(ω-carboxyheptadecanoyl)-des(B30) human insulin and B29-N^{ε}-(ω-carboxyheptadecanoyl) human insulin.

In addition, a variety of different insulin compositions are antidiabetics which should also be considered to fall within the scope of the present invention. For example this includes regular insulin, Semilente® insulin, isophane insulin, insulin zinc suspensions, protamine zinc insulin, and Ultralente® insulin.

Isophane insulin is an isophane mixture of protamine and insulin, wherein a ratio of protamine to insulin is mixed, which is equal to the ratio in a solution made by mixing equal parts of a solution of the two in which all the protamine precipitates and a solution of the two in which all the insulin precipitates.

In one embodiment insulin compositions according to the present invention are characterised by a fast onset of action, while in other embodiments the insulin compositions have a relatively slow onset but show a more or less prolonged action. Fast acting insulin compositions are usually solutions of insulin, while retarded acting insulin compositions can be suspen-

sions containing insulin in crystalline and/or amorphous form precipitated by addition of zinc salts alone or by addition of protamine or by a combination of both. In addition, some compositions have both a fast onset of action and a more prolonged action. Such a composition may be an insulin solution wherein protamine insulin crystals are suspended. Furthermore, compositions obtained by mixing an insulin solution with a suspension composition in the desired ratio are useful with the present invention.

The present invention preferably, may be used in connection with compositions comprising analogues and/or derivatives of human insulin. Thus, the insulin composition according to the invention may comprise one or more fast-acting analogues of human insulin, in particular analogues wherein the amino acid residue at position B28 is Asp, Lys, Leu, Val or Ala and the amino acid residue at position B29 is Lys or Pro; or des(B28-B30), des(B27) or des(B30) human insulin. The insulin analogue is preferably selected from analogues of human insulin wherein the amino acid residue at position B28 is Asp or Lys, and the amino acid residue at position B29 is Lys or Pro. The most preferred analogues are Asp_{B28} human insulin and Lys_{B28} Pro_{B29} human insulin.

In another embodiment the insulin composition according to the invention comprises an insulin derivative having a protracted profile of action, such an insulin having one or more lipophilic substituents. Lipophilic insulins may be acylated insulins, including those described in WO 95/07931, e.g. human insulin derivatives wherein the ε-amino group of Lys_{B29} contains an acyl substituent which comprises at least 6 carbon atoms.

In another embodiment of the present invention the antidiabetic belongs to the group of antidiabetica which can be administrated orally.

For example, the antidiabetic according to the present invention may be an orally active hypoglycemic agent. Orally active hypoglycemic agents preferably comprise sulfonylureas, biguanides, meglitinides, oxadiazolidinediones, thiazolidinediones, α-glucosidase inhibitors, glucagon antagonists such as those disclosed in WO 99/01423 and WO 00/39088 (Novo Nordisk A/S and Agouron Pharmaceuticals, Inc.), GLP-1 agonists such as those disclosed in WO 00/42026 (Novo Nordisk A/S and Agouron Pharmaceuticals, Inc.), potassium channel openers such as those disclosed in WO 97/26265, WO 99/03861 and WO 00/37474 (Novo Nordisk A/S), insulin sensitizers, PTPase inhibitors, inhibitors of hepatic enzymes involved in stimulation of gluconeogenesis and/or glycogenolysis, glucose uptake modulators, com-

pounds modifying the lipid metabolism such as antihyperlipidemic agents and antilipidemic agents, compounds lowering food intake, PPAR and RXR agonists and agents acting on the ATP-dependent potassium channel of the β-cells.

The group of biguanides decreases the blood sugar levels by inhibition of glucose uptake in the intestine, increase of peripheral glucose uptake and inhibition of glucose synthesis in the liver. The group for example comprises metformin.

The group of sulfonylureas stimulates the β-cells of the pancreas to produce more insulin. The group of sulfonylureas for example comprises glibenclamide, glicazide, acetohexamide, chlorpropamide, glimepiride, glipizide, glyburide, tolazamide and tolbutamide.

Alpha-glucosidase inhibitors may for example be selected from the group consisting of acarbose or miglitol.

Meglitinides may for example be selected from the group consisting of repaglinide, nateglinide or senaglinide.

Thiazolidinedione may for example be selected from the group consisting of pioglitazone, rosiglitazone, troglitazone, ciglitazone and the compounds disclosed in WO 97/41097, WO 97/41119, WO 97/41120, WO 00/41121 and WO 98/45202 (Dr. Reddy's Research Foundation).

Insulin sensitizers may for example be those disclosed in WO 99/19313, WO 00/50414, WO 00/63191, WO 00/63192, WO 00/63193 (Dr. Reddy's Research Foundation) and WO 00/23425, WO 00/23415, WO 00/23451, WO 00/23445, WO 00/23417, WO 00/23416, WO 00/63153, WO 00/63196, WO 00/63209, WO 00/63190 and WO 00/63189 (Novo Nordisk A/S).

Agents acting on the ATP-dependent potassium channel of the β-cells may for example be selected from the group consisting of tolbutamide, glibenclamide, glipizide, glicazide and repaglinide.

Preferably the oral antidiabetic is selected from the group consisting of tolbutamid, pioglitazone, rosiglitazone, glibenclamid, gliclazide, glipizide, acarbose, metformin and repaglinide.

DESCRIPTION OF THE INVENTION

The present invention demonstrates the possibility of administering inhibitors of the enzymes NEP and DPP-IV to individuals suffering from one or more of the following conditions: Diabetes, hypertension, chronic heart failure and fluid retentive states. Inhibition of the activity of these two enzymes will potentiate the insulin releasing activity of endogenous GLP-1 and other DPP-IV substrates like GIP.

The present invention demonstrates that there is an improved effect of administering both a DPP-IV inhibitor and a NEP inhibitor. Preferably, there is a synergistic effect of administering both a DPP-IV inhibitor and a NEP inhibitor.

Accordingly, it is a first objective of the present invention to provide treatment and prevention of one or more condition that may be regulated or normalised via inhibition of DPP-IV and NEP.

In one aspect of the invention, the condition is a metabolic disorder.

In another aspect of the invention, the condition is one in which blood glucose lowering is desired.

In another aspect of the invention, the condition is type 2 diabetes.

In another aspect of the invention, the condition is impaired glucose tolerance (IGT).

In another aspect of the invention, the condition is impaired fasting glucose (IFG).

In another aspect of the invention, the condition is hyperglycemia.

In another aspect of the invention, the condition is the progression of impaired glucose tolerance (IGT) to type 2 diabetes.

In another aspect of the invention, the condition is the progression of non-insulin requiring type 2 diabetes to insulin-requiring type 2 diabetes.

In another aspect of the invention, the condition is one in which increasing the number and/or the size of beta cells in a mammalian subject is desired.

In another aspect of the invention, the condition is beta cell degeneration, in particular apoptosis of beta cells.

In another aspect of the invention, the condition is disorders of food intake.

In another aspect of the invention, the condition is obesity.

In another aspect of the invention, the condition is one in which induction of satiety is desired.

In another aspect of the invention, the condition is dyslipidaemia.

In another aspect of the invention, the condition is functional dyspepsia, in particular irritable bowel syndrome.

In another aspect of the invention the condition is one requiring use of diuretic agents. These conditions include hypertension –essential hypertension, renovascular hypertension, hypertensive emergency, hypertension of endocrine cause, hypertension of neurogenic cause, as well as treatment and prevention of complications, worsening of the disease, pregnancy induced (e.g. pre-eclampsia). Further conditions are those in which chronic heart failure is treated, These include prevention of complications, prevention of deterioration/worsening of the disease – increase in survival rates of patients – e.g. one year survival rate.

In another aspect of the invention the condition is one in which treatment of associated disorders – fluid retention, swelling of the ankles, peripheral oedema, fatigue, dyspnoea, pulmonary oedema, emphysema, peripheral vascular disease, atherosclerosis, or intermittent claudication is desired.
In another aspect of the invention the condition is angina pectoris – e.g. angina on effort.
In another aspect of the invention the condition is re-occlusion of coronary arterial grafts.
In another aspect of the invention the condition is cerebrovascular stroke, ischaemic heart disease/ Myocardial infarction, valvular heart disease congenital heart disease, cardiomyopathy, or fluid retentive states.

In another aspect of the invention, it is contemplated to prevent hypoglycaemia in individuals treated with antidiabetics, comprising administering to said individual a combination of DPP-IV inhibitor and NEP inhibitor, or alternatively by administering a NEP inhibitor alone. A rise in the plasma concentration of glucagon by NEP inhibitors has been discovered. This may mean that NEP inhibitors alone, or in combination with DPP-IV inhibitors may result in a reduced incidence and severity of hypoglycaemia, hypoglycaemic unawareness when administered prophyactically in combination with antidiabetics.

According to the present invention, the inhibitors of DPP-IV and NEP are administered as a kit-of-parts. The kit-of-parts according to the present invention may be administrated in a manner, so that one or more components of the kit-of-parts are administrated by one route and another one or more components of the kit-of-parts are administrated by another route. By way of example, one component may be administrated orally, whereas another component may be administrated by subcutaneous injection.

Furthermore, the individual compounds of the kit-of-parts according to the present invention may be administered simultaneously, either as separate formulations or combined in a unit dosage form, or they may be administered sequentially.

The compounds according to the invention may also be administered with at least one additional compound.

The dosage requirements will vary with the particular drug composition employed, the route of administration and the particular individual being treated. Ideally, an individual to be treated by the present method will receive a pharmaceutically effective amount of the compound in the maximum tolerated dose, generally no higher than that required before drug resistance develops.

For all methods of use disclosed herein for the compounds, the daily oral dosage regimen will preferably be from about 0.01 to about 80 mg/kg of total body weight. The daily parenteral dosage regimen will preferably be from about 0.001 to about 80 mg/kg of total body weight. The daily topical dosage regimen will preferably be from 0.1 mg to 150 mg, administered one to four, preferably two or three times daily. The daily inhalation dosage regimen will preferably be from about 0.01 mg/kg to about 1 mg/kg per day. It will also be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of a compound or a pharmaceutically acceptable salt thereof will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular patient being treated, and that such optimums can be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, i.e., the number of doses of a compound or a pharmaceutically acceptable salt thereof given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

The term "unit dosage form" as used herein refers to physically discrete units suitable as unitary dosages for human and animal individuals, each unit containing a predetermined quantity of a compound, alone or in combination with other agents, calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier, or vehicle. The specifications for the unit dosage forms of the present invention de-

pend on the particular compound or compounds employed and the effect to be achieved, as well as the pharmacodynamics associated with each compound in the host. The dose administered should be an "effective amount" or an amount necessary to achieve an "effective level" in the individual patient.

Since the "effective level" is used as the preferred endpoint for dosing, the actual dose and schedule can vary, depending on interindividual differences in pharmacokinetics, drug distribution, and metabolism. The "effective level" can be defined, for example, as the blood or tissue level desired in the individual that corresponds to a concentration of one or more compounds according to the invention.

According to the present invention the DPP-IV inhibitor and NEP inhibitor may thus be administered in a regimen consisting of:

- Co-administration of DPP-IV and NEP inhibitors in separate formulations
- Sequential administration of DPP-IV and NEP inhibitors in separate formulations
- Administration of dual inhibitors, i.e. one compound that inhibits both DPP-IV and NEP
- Administration of formulations containing mixtures of DPP-IV inhibitors and NEP inhibitors
- Combinations of any one of the above with other diuretic agents, antidiabetics, other treatments for hypertension, chronic heart failure and fluid retentive states, such as digitalis inotropic agents, sympathomimetic agents, vasodilators, ACE inhibitors, angiotensin II receptor antagonists
- Combinations of any one of the above with other forms of therapy, e.g. diet or exercise

The conditions indicated above may thus be treated and/or prevented by using one or more of these regimens.

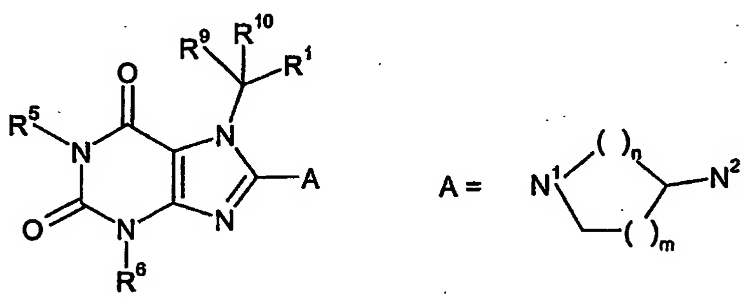
It is a further objective of the present invention to provide pharmaceutical preparations containing a DPP-IV inhibitor and a NEP inhibitor.

In one embodiment of the invention, the Dipeptidyl Peptidase-IV inhibitor to be applied in the present invention is selected from known inhibitors or prodrugs of such inhibitors, e.g. from

those disclosed in DD 296075 (Martin-Luther-Universität), WO 91/16339 and WO 93/08259 (New England Medical Centre Hospitals, Inc. and Tufts University School of Medicine), WO 95/15309, WO 01/40180, WO 01/81337 and WO 01/81304 (Ferring B.V.), WO 98/19998, US 6110949, WO 00/34241 and WO 01/96295 (Novartis AG), WO 99/46272 (Fondatech Benelux N.V.), WO 99/61431, WO 99/67278 ,WO 99/67279 and WO 01/14318 (Probiodrug Gesellschaft für Artzneimittelforschung Mbh.), WO 01/55105 (Novo Nordisk A/S) or WO 01/68603 (Bristol-Myers Squibb Co.).

In a preferred embodiment, the Dipeptidyl Peptidase-IV inhibitor to be applied in the present invention is (S)-1-[3-hydroxy-1-adamantyl)amino]acetyl-2-cyano-pyrrolidine or (S)-1-{2-[(5-cyanopyridin-2-yl)amino]ethyl-aminoacetyl}-2-cyano-pyrrolidine.

In another embodiment of the invention, the Dipeptidyl Peptidase-IV inhibitor to be applied in the present invention is a compound of formula I



Formula I

wherein A may be attached at either N^1 or at N^2 to the purine system and each n and m is one or two independently

R^1 is aryl optionally substituted with one or more R^2 independently or heteroaryl optionally substituted with one or more R^2 independently;

R^2 is H; C_1-C_7 alkyl; C_2-C_7 alkenyl; C_2-C_7 alkynyl; C_3-C_7 cycloalkyl; C_3-C_7 cycloheteroalkyl; -NHCOR^3; -NHSO$_2$R^3; -SR^3; -SOR^3; -SO$_2$R^3; -OCOR^3; -CO$_2$R^4; -CON(R^4)$_2$; -CSN(R^4)$_2$; -NHCON(R^4)$_2$; -NHCSN(R^4)$_2$; -NHCONHH$_2$; -SO$_2$N(R^4)$_2$; -OR^4; cyano; nitro; halogen, wherein each alkyl, alkenyl, alkynyl, cycloalkyl and cycloheteroalkyl is optionally substituted with one or more R^3 independently;

R^3 is C_1-C_{10} alkyl; C_2-C_{10} alkenyl; C_2-C_{10} alkynyl; C_3-C_7 cycloalkyl; aryl; heteroaryl; OR^{11}; $N(R^{11})_2$; SR^{11}, wherein each alkyl, alkenyl, alkynyl, cycloalkyl, aryl and heteroaryl is substituted with one or more R^{11} independently;

R^4 is H; C_1-C_{10} alkyl; C_2-C_{10} alkenyl; C_2-C_{10} alkynyl; C_3-C_7 cycloalkyl; C_3-C_7 cycloheteroalkyl; aryl; aryl-C_1-C_5 alkyl; heteroaryl; heteroaryl-C_1-C_5 alkyl, wherein each alkyl, alkenyl, alkynyl, cycloalkyl, cycloheteroalkyl, aryl, aryl-C_1-C_5 alkyl, heteroaryl, and heteroaryl-C_1-C_5 alkyl is substituted with one or more R^{11} independently;

R^5 is H; C_1-C_{10} alkyl; C_2-C_{10} alkenyl; C_2-C_{10} alkynyl; C_3-C_7 cycloalkyl; C_3-C_7 cycloalkyl-C_1-C_5 alkyl; C_3-C_7 cycloheteroalkyl; C_3-C_7 cycloheteroalkyl-C_1-C_5 alkyl; aryl; heteroaryl; aryl-C_1-C_5 alkyl; heteroaryl-C_1-C_5 alkyl; -OR^7; -$[(CH_2)_o$-$O]_p$-alkyl, wherein o and p are 1-3 independently, and wherein each alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-C_1-C_5 alkyl, cycloheteroalkyl, C_3-C_7 cycloheteroalkyl-C_1-C_5 alkyl, aryl, aryl-C_1-C_5 alkyl, heteroaryl, aryl-C_1-C_5 alkyl, and heteroaryl-C_1-C_5 alkyl is optionally substituted with one or more R^7 independently;

R^6 is C_1-C_{10} alkyl; C_2-C_{10} alkenyl; C_2-C_{10} alkynyl; C_3-C_7 cycloalkyl; C_3-C_7 cycloheteroalkyl; aryl; heteroaryl; aryl-C_1-C_5 alkyl; heteroaryl-C_1-C_5 alkyl; C_3-C_7 cycloheteroalkyl-C_1-C_5 alkyl, wherein each alkyl, alkenyl, alkynyl, cycloalkyl, cycloheteroalkyl, C_3-C_7 cycloheteroalkyl-C_1-C_5 alkyl, aryl, aryl-C_1-C_5 alkyl, heteroaryl, and heteroaryl-C_1-C_5 alkyl is optionally substituted with one or more R^{11} independently;

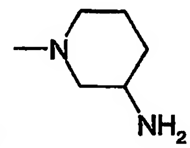
R^7 is H; =O; C_1-C_{10} alkyl; C_2-C_{10} alkenyl; C_2-C_{10} alkynyl; C_3-C_7 cycloalkyl; C_3-C_7 cycloheteroalkyl; aryl; heteroaryl, OR^{11}; $N(R^{11})_2$; SR^{11}, wherein each alkyl, alkenyl, alkynyl, cycloalkyl, cycloheteroalkyl, aryl, and heteroaryl is optionally substituted with one or more R^{11} independently;

R^8 is C_1-C_{10} alkyl; C_2-C_{10} alkenyl; C_2-C_{10} alkynyl; C_3-C_7 cycloalkyl; C_3-C_7 cycloheteroalkyl; aryl; heteroaryl, OR^{11}; $N(R^{11})_2$; SR^{11}, wherein each alkyl, alkenyl, alkynyl, cycloalkyl, cycloheteroalkyl, aryl, and heteroaryl is optionally substituted with one or more R^{11} independently;

R^9 and R^{10} is independently H, C_1-C_{10} alkyl optionally substituted with one or more R^8 independently, halogen;

R^{11} is H; -CF_3; -CCl_3; -OCF_3; -OMe; cyano; halogen; -OH, COMe; -$CONH_2$; CONHMe; $CONMe_2$; -NO_2;

if R^9 and R^{10} is C_1-C_{10} alkyl they may be connected to form a cyclopropyl ring;
if two R^4 or two R^{11} are attached to the same nitrogen they may be connected to form a 3- to 7-membered ring;
or a salt thereof with a pharmaceutically acceptable acid or base.

In a further embodiment of the compounds of formula I A is 

In a further embodiment of the compounds of formula I R^1 is aryl optionally substituted with one or more R^2 independently.
In a further embodiment of the compounds of formula I R^1 is aryl.
In a further embodiment of the compounds of formula I R^1 is phenyl.
In a further embodiment of the compounds of formula I R_2 is C_1-C_7 alkyl; C_2-C_7 alkynyl; cyano; or halogen, wherein each alkyl and alkynyl is optionally substituted with one or more R^3 independently.
In a further embodiment of the compounds of formula I R_2 is C_1-C_7 alkyl; C_2-C_7 alkynyl; cyano; or halogen.
In a further embodiment of the compounds of formula I R_2 is cyano or halogen.
In a further embodiment of the compounds of formula I R^3 is C_1-C_{10} alkyl or aryl, wherein each alkyl or aryl is substituted with one or more R^{11} independently.
In a further embodiment of the compounds of formula I R^3 is C_1-C_{10} alkyl or aryl.
In a further embodiment of the compounds of formula I R^3 is methyl or phenyl.
In a further embodiment of the compounds of formula I R^4 is H; C_1-C_{10} alkyl or aryl, wherein each alkyl or aryl is substituted with one or more R^{11} independently.
In a further embodiment of the compounds of formula I R^4 is H; C_1-C_{10} alkyl or aryl.
In a further embodiment of the compounds of formula I R^4 is H, methyl or phenyl.
In a further embodiment of the compounds of formula I R^5 is H; C_1-C_{10} alkyl; aryl-C_1-C_6 alkyl; or heteroaryl-C_1-C_6 alkyl, wherein each alkyl, aryl-C_1-C_6 alkyl and heteroaryl-C_1-C_6 alkyl is optionally substituted with one or more R^7 independently.
In a further embodiment of the compounds of formula I R^5 is H or C_1-C_{10} alkyl optionally substituted with one or more R^7 independently.
In a further embodiment of the compounds of formula I R^5 is H or C_1-C_{10} alkyl.
In a further embodiment of the compounds of formula I R^5 is H.
In a further embodiment of the compounds of formula I R^5 is methyl.

In a further embodiment of the compounds of formula I R^6 is C_1-C_{10} alkyl; aryl-C_1-C_5 alkyl; or heteroaryl-C_1-C_5 alkyl, wherein each alkyl, aryl-C_1-C_5 alkyl and heteroaryl-C_1-C_5 alkyl is optionally substituted with one or more R^{11} independently.

In a further embodiment of the compounds of formula I R^6 is C_1-C_{10} alkyl; aryl-C_1-C_5 alkyl; or heteroaryl-C_1-C_5 alkyl.

In a further embodiment of the compounds of formula I R^6 is C_1-C_{10} alkyl optionally substituted with one or more R^{11} independently.

In a further embodiment of the compounds of formula I R^6 is C_1-C_{10} alkyl.

In a further embodiment of the compounds of formula I R^6 is methyl.

In a further embodiment of the compounds of formula I R^7 is H; =O; aryl; heteroaryl, OR^{11}; $N(R^{11})_2$; SR^{11}, wherein each aryl and heteroaryl is optionally substituted with one or more R^{11} independently.

In a further embodiment of the compounds of formula I R^7 is H; =O; aryl; or heteroaryl.

In a further embodiment of the compounds of formula I R^7 is H; =O; OR^{11}; $N(R^{11})_2$; or SR^{11}.

In a further embodiment of the compounds of formula I R^7 is H or =O.

In a further embodiment of the compounds of formula I R^8 is aryl or heteroaryl, wherein each aryl and heteroaryl is optionally substituted with one or more R^{11} independently.

In a further embodiment of the compounds of formula I R^8 is aryl or heteroaryl.

In a further embodiment of the compounds of formula I R^8 is phenyl.

In a further embodiment of the compounds of formula I R^9 is H; C_1-C_{10} alkyl; or halogen.

In a further embodiment of the compounds of formula I R^9 is H.

In a further embodiment of the compounds of formula I R^{10} is H; C_1-C_{10} alkyl; or halogen.

In a further embodiment of the compounds of formula I R^{10} is H.

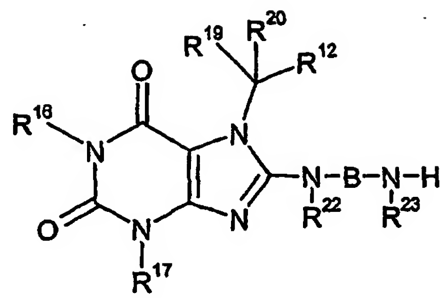
In a further embodiment of the compounds of formula I R^{11} is cyano; halogen; -CONHMe; or -$CONMe_2$.

In a further embodiment of the compounds of formula I R^{11} is cyano or halogen.

In a further embodiment of the compounds of formula I n is one.

In a further embodiment of the compounds of formula I m is one.

In another embodiment of the invention, the Dipeptidyl Peptidase-IV inhibitor to be applied in the present invention is a compound of formula II



Formula II

wherein

B is C_2-C_8 alkylene; C_2-C_{10} alkenylene; C_3-C_7 cycloalkylene; C_3-C_7 cycloheteroalkylene; arylene; heteroarylene; C_1-C_2 alkylene-arylene; arylene-C_1-C_2 alkylene; C_1-C_2 alkylene-arylene-C_1-C_2 alkylene, wherein each alkylene, alkenylene, cycloalkylene, cycloheteroalkylene, arylene, or heteroarylene is optionally substituted with one or more R^{14} independently;

R^{12} is aryl optionally substituted with one or more R^{13} independently or heteroaryl optionally substituted with one or more R^{13} independently;

R^{13} is H; C_1-C_7 alkyl; C_2-C_7 alkenyl; C_2-C_7 alkynyl; C_3-C_7 cycloalkyl; C_3-C_7 cycloheteroalkyl; -$NHCOR^{14}$; -$NHSO_2R^{14}$; -SR^{14}; -SOR^{14}; -SO_2R^{14}; -$OCOR^{14}$; -CO_2R^{15}; -$CON(R^{15})_2$; -$CSN(R^{15})_2$; -$NHCON(R^{15})_2$; -$NHCSN(R^{15})_2$; -$NHCONNH_2$; -$SO_2N(R^{15})_2$; -OR^{15}; cyano; nitro; halogen, wherein each alkyl, alkenyl, alkynyl, cycloalkyl and cycloheteroalkyl is optionally substituted with one or more R^{14} independently;

R^{14} is C_1-C_{10} alkyl; C_2-C_{10} alkenyl; C_2-C_{10} alkynyl; C_3-C_7 cycloalkyl; aryl; heteroaryl; OR^{21}; $N(R^{21})_2$; SR^{21}, wherein each alkyl, alkenyl, alkynyl, cycloalkyl, aryl and heteroaryl is optionally substituted with one or more R^{21} independently;

R^{15} is H; C_1-C_{10} alkyl; C_2-C_{10} alkenyl; C_2-C_{10} alkynyl; C_3-C_7 cycloalkyl; C_3-C_7 cycloheteroalkyl; aryl; aryl-C_1-C_5 alkylene; heteroaryl; heteroaryl-C_1-C_5 alkylene, wherein each alkyl, alkenyl, alkynyl, cycloalkyl, cycloheteroalkyl, aryl, aryl-C_1-C_5 alkylene, heteroaryl, and heteroaryl-C_1-C_5 alkylene is optionally substituted with one or more R^{21} independently;

R^{16} is H; C_1-C_{10} alkyl; C_2-C_{10} alkenyl; C_2-C_{10} alkynyl; C_3-C_7 cycloalkyl; C_3-C_7 cycloheteroalkyl; aryl; heteroaryl; -OR^{18}; -$[(CH_2)_o$-$O]_p$-C_1-C_5 alkyl, wherein o and p are 1-3 independently, and

wherein each alkyl, alkenyl, alkynyl, cycloalkyl, cycloheteroalkyl, aryl, and heteroaryl is optionally substituted with one or more R^{18} independently;

R^{17} is H; C_1-C_{10} alkyl; C_2-C_{10} alkenyl; C_2-C_{10} alkynyl; C_3-C_7 cycloalkyl; C_3-C_7 cycloheteroalkyl; aryl; heteroaryl; aryl-C_1-C_6 alkylene; heteroaryl-C_1-C_6 alkylene; C_3-C_7 cycloheteroalkyl-C_1-C_6 alkylene, wherein each alkyl, alkenyl, alkynyl, cycloalkyl, cycloheteroalkyl, C_3-C_7 cycloheteroalkyl-C_1-C_6 alkylene, aryl, aryl-C_1-C_6 alkylene, heteroaryl, aryl-C_1-C_6 alkylene, and heteroaryl-C_1-C_6 alkylene is optionally substituted with one or more R^{21} independently;

R^{18} is H; =O; C_1-C_{10} alkyl; C_2-C_{10} alkenyl; C_2-C_{10} alkynyl; C_3-C_7 cycloalkyl; C_3-C_7 cycloheteroalkyl; aryl; heteroaryl, OR^{21}; $N(R^{21})_2$; SR^{21}; cyano; hydroxy; halogen; $-CF_3$; $-CCl_3$; $-OCF_3$; or $-OCH_3$ wherein each alkyl, alkenyl, alkynyl, cycloalkyl, cycloheteroalkyl, aryl, and heteroaryl is optionally substituted with one or more R^{21} independently;

R^{19} is H; C_1-C_{10} alkyl; C_2-C_{10} alkenyl; C_2-C_{10} alkynyl; C_3-C_7 cycloalkyl; C_3-C_7 cycloheteroalkyl; aryl; heteroaryl, OR^{21}; $N(R^{21})_2$; SR^{21}, wherein each alkyl, alkenyl, alkynyl, cycloalkyl, cycloheteroalkyl, aryl, and heteroaryl is optionally substituted with one or more R^{21} independently;

R^{20} is H; C_1-C_{10} alkyl optionally substituted with one or more R^{19} independently; or halogen;

R^{21} is H; $-CF_3$; $-CCl_3$; $-OCF_3$; $-OCH_3$; cyano; halogen; -OH, $-COCH_3$; $-CONH_2$; $-CONHCH_3$; $-CON(CH_3)_2$; $-NO_2$; $-SO_2NH_2$; or $-SO_2N(CH_3)_2$;

if two R^{15} or two R^{21} are attached to the same nitrogen they may be connected to form a 3- to 7-membered ring;

R^{22} is H; C_1-C_6 alkyl optionally substituted with one or more R^{14} independently;

R^{23} is H; C_1-C_6 alkyl optionally substituted with one or more R^{14} independently; or if B is C_3-C_7 cycloalkylene or C_3-C_7 cycloheteroalkylene R^{23} may be a valence bond between the nitrogen to which R^{23} is attached and one of the atoms in the cycloalkylene or cycloheteroalkylene;

or a salt thereof with a pharmaceutically acceptable acid or base.

In another embodiment of the compounds of formula II B is C_2-C_8 alkylene; C_2-C_{10} alkenylene; C_3-C_7 cycloalkylene; C_3-C_7 cycloheteroalkylene; or arylene, wherein each alkylene, alkenylene, cycloalkylene, cycloheteroalkylene, or arylene is optionally substituted with one or more R^{14} independently;

In another embodiment of the compounds of formula II B is C_3-C_7 cycloalkylene optionally substituted with one or more R^{14} independently.

In another embodiment of the compounds of formula II B is cyclohexylene optionally substituted with one or more R^{14} independently.

In another embodiment of the compounds of formula II B is cyclohexylene.

In another embodiment of the compounds of formula II R^{12} is aryl optionally substituted with one or more R^{13} independently.

In another embodiment of the compounds of formula II R^{12} is phenyl optionally substituted with one or more R^{13} independently.

In another embodiment of the compounds of formula II R^{13} is C_1-C_7 alkyl; C_2-C_7 alkynyl; cyano; or halogen, wherein each alkyl and alkynyl is optionally substituted with one or more R^{14} independently.

In another embodiment of the compounds of formula II R^{13} is C_1-C_7 alkyl; C_2-C_7 alkynyl; cyano; or halogen.

In another embodiment of the compounds of formula II R^{13} is halogen.

In another embodiment of the compounds of formula II R^{14} is C_1-C_{10} alkyl or aryl, wherein each alkyl or aryl is substituted with one or more R^{21} independently.

In another embodiment of the compounds of formula II R^{14} is C_1-C_{10} alkyl or aryl.

In another embodiment of the compounds of formula II R^{14} is methyl or phenyl.

In another embodiment of the compounds of formula II R^{15} is H; C_1-C_{10} alkyl or aryl, wherein each alkyl or aryl is substituted with one or more R^{21} independently.

In another embodiment of the compounds of formula II R^{15} is H; C_1-C_{10} alkyl or aryl.

In another embodiment of the compounds of formula II R^{15} is H, methyl or phenyl.

In another embodiment of the compounds of formula II R^{16} is H; C_1-C_{10} alkyl; aryl-C_1-C_5 alkylene; or heteroaryl-C_1-C_6 alkylene, wherein each alkyl, aryl-C_1-C_6 alkylene and heteroaryl-C_1-C_5 alkylene is optionally substituted with one or more R^{18} independently.

In another embodiment of the compounds of formula II R^{16} is H; C_1-C_{10} alkyl optionally substituted with one or more R^{18} independently; or C_2-C_{10} alkenyl optionally substituted with one or more R^{18} independently.

In another embodiment of the compounds of formula II R^{16} is H or C_1-C_{10} alkyl optionally substituted with one or more R^{18} independently.

In another embodiment of the compounds of formula II R^{16} is H.

In another embodiment of the compounds of formula II R^{16} is methyl or ethyl optionally substituted with one or more R^{18} independently.

In another embodiment of the compounds of formula II R^{17} is C_1-C_{10} alkyl; aryl-C_1-C_5 alkylene; or heteroaryl-C_1-C_5 alkylene, wherein each alkyl, aryl-C_1-C_5 alkylene and heteroaryl-C_1-C_5 alkylene is optionally substituted with one or more R^{21} independently.

In another embodiment of the compounds of formula II R^{17} is C_1-C_{10} alkyl; aryl-C_1-C_5 alkylene; or heteroaryl-C_1-C_5 alkylene.

In another embodiment of the compounds of formula II R^{17} is C_1-C_{10} alkyl optionally substituted with one or more R^{21} independently.

In another embodiment of the compounds of formula II R^{17} is C_1-C_{10} alkyl.

In another embodiment of the compounds of formula II R^{17} is methyl or ethyl optionally substituted by one or more R^{21} independently.

In another embodiment of the compounds of formula II R^{18} is H; =O; C_1-C_{10} alkyl; C_3-C_7 cycloalkyl; C_3-C_7 cycloheteroalkyl; aryl; heteroaryl, OR^{21}; $N(R^{21})_2$; SR^{21}, wherein each alkyl, cycloalkyl, cycloheteroalkyl, aryl, and heteroaryl is optionally substituted with one or more R^{21} independently.

In another embodiment of the compounds of formula II R^{18} is =O; C_3-C_7 cycloalkyl; C_3-C_7 cycloheteroalkyl; aryl; or heteroaryl, wherein each cycloalkyl, cycloheteroalkyl, aryl, and heteroaryl is optionally substituted with one or more R^{21} independently.

In another embodiment of the compounds of formula II R^{18} is =O; C_3-C_7 cycloalkyl optionally substituted with one or more R^{21} independently or aryl optionally substituted with one or more R^{21} independently.

In another embodiment of the compounds of formula II R^{18} is =O or aryl optionally substituted with one or more R^{21} independently.

In another embodiment of the compounds of formula II R^{18} is =O or phenyl optionally substituted by one or more R^{21} independently.

In another embodiment of the compounds of formula II R^{19} is aryl or heteroaryl, wherein each aryl and heteroaryl is optionally substituted with one or more R^{21} independently.

In another embodiment of the compounds of formula II R^{19} is aryl or heteroaryl.

In another embodiment of the compounds of formula II R^{19} is phenyl.

In another embodiment of the compounds of formula II R^{20} is H; C_1-C_{10} alkyl; or halogen.

In another embodiment of the compounds of formula II R^{20} is H.

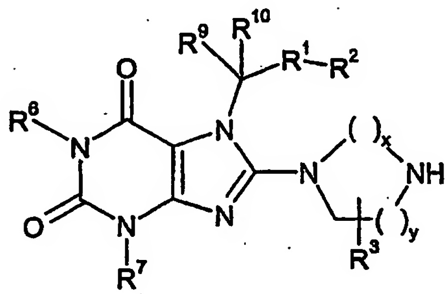
In another embodiment of the compounds of formula II R^{21} is H; $-CF_3$; -OH; cyano; halogen; $-OCF_3$; or $-OCH_3$.

In another embodiment of the compounds of formula II R^{21} is H; cyano; halogen; or $-OCH_3$.

In another embodiment of the compounds of formula II R^{22} is H.

In another embodiment of the compounds of formula II R^{23} is H.

In another embodiment of the invention, the Dipeptidyl Peptidase-IV inhibitor to be applied in the present invention is a compound of formula III



Formula III

wherein

x and y are one or two independently

R^1 is C=O; C=S; C_1-C_2 alkyl optionally substituted with one or more R^4 independently; C_2 alkenyl substituted with one or more R^4 independently; C_2 alkynyl; C_3-C_7 cycloalkyl optionally substituted with one or more R^4 independently; C_3-C_7 cycloheteroalkyl optionally substituted with one or more R^4 independently; aryl optionally substituted with one or more R^4 independently; aryl C_1-C_3 alkyl optionally substituted with one or more R^4 independently; heteroaryl optionally substituted with one or more R^4 independently; heteroaryl C_1-C_3 alkyl optionally substituted with one or more R^4 independently; perhalo C_1-C_{10} alkyl; perhalo C_1-C_{10} alkyloxy;

R^2 is H; C_1-C_7 alkyl optionally substituted with one or more R^4 independently; C_2-C_7 alkenyl optionally substituted with one or more R^4 independently; C_2-C_7 alkynyl optionally substituted with one or more R^4 independently; C_3-C_7 cycloalkyl optionally substituted with one or more R^4 independently; C_3-C_7 cycloheteroalkyl optionally substituted with one or more R^4 independently; aryl optionally substituted with one or more R^4 independently; aryl C_1-C_3 alkyl optionally substituted with one or more R^4 independently; heteroaryl C_1-C_3 alkyl optionally substituted with one or more R^4 independently; heteroaryl optionally substituted with one or more R^4 independently, -SH; $-SR^5$; SOR^5; SO_2R^5; -CHO; $-CH(OR^5)_2$; carboxy; $-CO_2R^4$;

NHCONNH$_2$; -NHCSNH$_2$; -NHCONH$_2$; -NHCOR^4; -NHSO$_2$R^5; -O-CO-(C$_1$-C$_6$) alkyl optionally substituted with one or more R^4 independently; cyano; nitro; halogen; hydroxy; perhalo C$_1$-C$_7$ alkyl; perhalo C$_1$-C$_7$ alkyloxy; -SO$_2$NH$_2$; -SO$_2$NH(R^5); -SO$_2$(R^5)$_2$; -CONH$_2$; -CSNH$_2$; -CON$_2$H$_3$; -CONH(R^5); -CON(R^5)$_2$; C$_1$-C$_{10}$ alkyloxy optionally substituted with R^4 independently; C$_2$-C$_{10}$ alkenyloxy optionally substituted with R^4; C$_2$-C$_{10}$ alkynyloxy optionally substituted with R^4 independently, aryloxy optionally substituted with R^4 independently; heteroaryloxy optionally substituted with R^4 independently;

R^3 is H; C$_1$-C$_{10}$ alkyl optionally substituted with one or more R^4 independently; C$_2$-C$_{10}$ alkenyl optionally substituted with one or more R^4 independently; C$_2$-C$_{10}$ alkynyl optionally substituted with one or more R^4 independently; C$_3$-C$_7$ cycloalkyl optionally substituted with one or more R^4 independently; C$_3$-C$_7$ cycloheteroalkyl optionally substituted with one or more R^4 independently; aryl optionally substituted with one or more R^4 independently; aryl C$_1$-C$_3$ alkyl optionally substituted with one or more R^4 independently; heteroaryl C$_1$-C$_3$ alkyl optionally substituted with one or more R^4 independently; heteroaryl optionally substituted with one or more R^4 independently; C$_1$-C$_{10}$ alkyl-NH(CH$_2$)$_{1-4}$NH-aryl optionally substituted with one or more R^4 independently; C$_1$-C$_{10}$ alkyl-NH(CH$_2$)$_{1-4}$NH-heteroaryl optionally substituted with one or more R^4 independently; C$_1$-C$_{10}$ alkyl-O(CH$_2$)$_{1-4}$NH-aryl optionally substituted with one or more R^4 independently; C$_1$-C$_{10}$ alkyl-O(CH$_2$)$_{1-4}$NH-heteroaryl optionally substituted with one or more R^4 independently; C$_1$-C$_{10}$ alkyl-O(CH$_2$)$_{1-4}$O-aryl optionally substituted with one or more R^4 independently; C$_1$-C$_{10}$ alkyl-O(CH$_2$)$_{1-4}$O-heteroaryl optionally substituted with one or more R^4 independently; C$_1$-C$_{10}$ alkyl-S(CH$_2$)$_{1-4}$NH-aryl optionally substituted with one or more R^4 independently; C$_1$-C$_{10}$ alkyl-S(CH$_2$)$_{1-4}$NH-heteroaryl optionally substituted with one or more R^4 independently; C$_1$-C$_{10}$ alkyl-S(CH$_2$)$_{1-4}$S-aryl optionally substituted with one or more R^4 independently; C$_1$-C$_{10}$ alkyl-S(CH$_2$)$_{1-4}$S-heteroaryl optionally substituted with one or more R^4 independently; C$_1$-C$_{10}$ alkyl-O-C$_1$-C$_6$alkyl optionally substituted with one or more R^4; -NHCOR^4; -NHSO$_2$R^5; -O-CO-(C$_1$-C$_6$) alkyl optionally substituted with one or more R^4 independently; -SH; -SR^5; -SOR^5; -SO$_2$R^5; -CHO; -CH(OR^5)$_2$; carboxy; cyano; nitro; halogen; hydroxy; -SO$_2$NH$_2$; -SO$_2$NH(R^5); -SO$_2$N(R^5)$_2$; -CONH$_2$; -CONH(R^5); -CON(R^5)$_2$; -CSNH$_2$; -CONHNH$_2$; -CO2R^4; -NHCNHNH$_2$; -NHCSNH$_2$; -NHCONH$_2$;

R^4 is C$_1$-C$_{10}$ alkyl optionally substituted with one or more R^8 independently; C$_2$-C$_{10}$ alkenyl optionally substituted with one or more R^8 independently; C$_2$-C$_{10}$ alkynyl optionally substituted with one or more R^8 independently; C$_3$-C$_7$ cycloalkyl optionally substituted with one or more R^8 independently; C$_3$-C$_7$ cycloheteroalkyl optionally substituted with one or more R^8 inde-

pendently; aryl optionally substituted with one or more R^8 independently; heteroaryl optionally substituted with one or more R^8 independently; amino; amino substituted with one or more C_1-C_{10} alkyl optionally substituted with one or more R^8; amino substituted with one or two aryl optionally substituted with one or more R^8 independently; heteroaryl optionally substituted with one or more R^8 independently; =O; =S; -CO-R5; -COOR5; -O-CO-(C_1-C_6) alkyl optionally substituted with one or more R^8 independently; $NH(CH_2)_{1\text{-}4}NH$-aryl; $NH(CH_2)_{1\text{-}4}NH$-heteroaryl; -$NHCOR^5$; -SOR^5; SO_2R^5; carboxy; cyano; N-hydroxyimino; nitro; halogen; hydroxy; perhalo C_1-C_{10} alkyl; perhalo C_1-C_{10} alkyloxy; -SH; -SR^5; -SO_3H; -SO_3R^5; -SO_2R^5; -SO_2NH_2; -$SO_2NH(R^5)$; -$SO_2N(R^5)_2$; -$CONH_2$; -$CONH(R^5)$; -$CON(R^5)_2$; C_1-C_{10} alkyloxy optionally substituted with one or more R^8 independently; C_2-C_{10} alkenyloxy optionally substituted with one or more R^8 independently; C_2-C_{10} alkynyloxy optionally substituted with one or more R^8 independently; aryloxy optionally substituted with one or more R^8 independently; heteroaryloxy optionally substituted with one or more R^8 independently;

and two R^4 attached to the same carbon atom may form a spiroheterocyclic system, preferably hydantoin; thiohydantoin; oxazolidine-2,5-dione;

R^5 is C_1-C_{10} alkyl optionally substituted with one or more R^8 independently; C_2-C_{10} alkenyl optionally substituted with one or more R^8 independently; C_2-C_{10} alkynyl optionally substituted with one or more R^8 independently; C_3-C_7 cycloalkyl optionally substituted with one or more R^8 independently; C_3-C_7 cycloheteroalkyl optionally substituted with one or more R^8 independently; aryl optionally substituted with one or more R^8 independently; aryl C_1-C_6 alkyl optionally substituted with one or more R^8 independently; heteroaryl optionally substituted with one or more R^8 independently; heteroaryl C_1-C_6 alkyl optionally substituted with one or more R^8 independently;

R^6 is H; C_1-C_{10} alkyl optionally substituted with one or more R^4 independently; C_2-C_{10} alkenyl optionally substituted with one or more R^4 independently; C_2-C_{10} alkynyl optionally substituted with one or more R^4 independently; C_3-C_7 cycloalkyl optionally substituted with one or more R^4 independently; C_3-C_7 cycloheteroalkyl optionally substituted with one or more R^4 independently; aryl optionally substituted with one or more R^4 independently; heteroaryl optionally substituted with one or more R^4 independently;

R^7 is H; C_1-C_{10} alkyl optionally substituted with one or more R^4 independently; C_2-C_{10} alkenyl optionally substituted with one or more R^4 independently; C_2-C_{10} alkynyl optionally substituted with one or more R^4 independently; C_3-C_7 cycloalkyl optionally substituted with one or more

R^4 independently; C_3-C_7 cycloheteroalkyl optionally substituted with one or more R^4 independently; aryl optionally substituted with one or more R^4 independently; heteroaryl optionally substituted with one or more R^4 independently;

R^8 is H, amidoxime; nitro, tetrazole; pentafluorophenyl; $-CH_2OH$; -CHO; $-C(OCH_3)_2$; $-COCH_3$; $-CF_3$; $-CCl_3$; $-OCF_3$; $-OCH_3$; -CN; $-CO_2H$; $-CO_2CH_3$; $-CONH_2$; $-CSNH_2$; $-CON_2H_3$; $-SO_3H$; $-SO_2NH_2$; $-SO_2NHCH_3$; $-SO_2N(CH_3)_2$; $-SO_2$ (1-piperazinyl);$-SO_2$ (4-methylpiperazin-1-yl); $-SO_2$ (pyrrolidin-1-yl); $-SO_2$ (piperidin-1-yl); $-SO_2$ (morpholin-4-yl); N-hydroxyimino; $-NH_2$; $-NHCH_3$; $-N(CH_3)_2$; $-NHCNHNH_2$; $-NHCNHNHCH_3$; $-NHCSNH_2$; $-NHCSNHCH_3$; $-NHCONH_2$; $-NHCONHCH_3$; $-NHCOCH_3$; $-NHSO_2CH_3$; piperazinyl; morhpolin-4-yl; thiomorpholin-4-yl; pyrrolidin-1-yl; piperidin-1-yl; halogen; -OH; -SH; $-SCH_3$; -aminoacetyl; $-OPO_3H$; $-OPO_2OCH_3$; $-PO_3H_2$; $-PO(OCH_3)_2$; $PO(OH)(OCH_3)$;

R^9 is H; halogen; C_1-C_{10} alkyl optionally substituted with one or more R^4 independently

R^{10} is H; halogen;

or, R^9 and R^{10} may be connected to form a cyclopropyl ring;

or a salt thereof with a pharmaceutically acceptable acid or base;

with the exception of the following compounds:

1,3-dimethyl-7-(2-oxo-propyl) -8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,

1,3,1',3',7'-pentamethyl-8-piperazin-1-yl-3,7,3',7'-tetrahydro-7,8'-methanediyl-bis-purine-2,6-dione,

3,4,5-trimethoxy-benzoic acid 2-(1,3-dimethyl-2,6-dioxo-8-piperazin-1-yl-1,2,3,6-tetrahydro-purin-7-yl) -ethyl ester,

7-[2-Hydroxy-3-(4-methoxy-phenoxy) -propyl]-3-methyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,

7-[2-hydroxy-2-(4-nitro-phenyl) -ethyl]-3-methyl-8-piperazin-1-yl-3,7,8,9-tetrahydro-purine-2,6-dione,

7-Benzyl-3-methyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,

7-(4-Chloro-benzyl) -3-methyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,

7-(2-Chloro-benzyl) -3-methyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,

7-Ethyl-3-methyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,

3-Methyl-8-piperazin-1-yl-1,7-dipropyl-3,7-dihydro-purine-2,6-dione,
3-Methyl-7-(3-methyl-butyl) -8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,
7-Butyl-3-methyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,
3-Methyl-7-(3-phenyl-propyl) -8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,
7-But-2-enyl-3-methyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,
7-(3-Chloro-but-2-enyl) -3-methyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,
7-Heptyl-3-methyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,
3-Methyl-7-(1-phenyl-ethyl) -8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,
3-Methyl-7-(3-methyl-benzyl) -8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,
3-Methyl-7-propyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione, and
3-Methyl-7-pentyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione.

In another embodiment of the compounds of formula III, R^1 is C_2 alkenyl optionally substituted with one or more R^4 independently or aryl-C_1-C_3 alkyl optionally substituted with one or more R^4 independently.

In another embodiment of the compounds of formula III, R^2 is H, C_1-C_7 alkyl optionally substituted with one or more R^4 independently, cyano, nitro, or halogen.

In another embodiment of the compounds of formula III, R^4 is C_1-C_{10} alkyl optionally substituted with one or more R^8 independently; C_2-C_{10} alkenyl optionally substituted with one or more R^8 independently; C_2-C_{10} alkynyl optionally substituted with one or more R^8 independently; C_3-C_7 cycloalkyl optionally substituted with one or more R^8 independently; aryl optionally substituted with one or more R^8 independently; heteroaryl optionally substituted with one or more R^8 independently; amino; amino substituted with one or more C_1-C_{10} alkyl optionally substituted with one or more R^8; amino substituted with one or two aryl optionally substituted with one or more R^8 independently; heteroaryl optionally substituted with one or more R^8 independently; =O; =S; -CO-R^5; -COOR^5, carboxy; cyano; nitro; halogen; hydroxy; -SH; -SR^5; -CONH_2; -CONH(R^5); -CON(R^5)$_2$; C_1-C_{10} alkyloxy optionally substituted with one or more R^8 independently; C_2-C_{10} alkenyloxy optionally substituted with one or more R^8 independently; aryloxy optionally substituted with one or more R^8 independently; heteroaryloxy optionally substituted with one or more R^8 independently;
and two R^4 attached to the same carbon atom may form a spiroheterocyclic system, preferably hydantoin; thiohydantoin; oxazolidine-2,5-dione

In another embodiment of the compounds of formula III, R^4 is C_1-C_{10} alkyl optionally substituted with one or more R^8 independently; C_2-C_{10} alkenyl optionally substituted with one or more R^8 independently; C_2-C_{10} alkynyl optionally substituted with one or more R^8 independend-

ently; C_3-C_7 cycloalkyl optionally substituted with one or more R^8 independently; aryl optionally substituted with one or more R^8 independently; heteroaryl optionally substituted with one or more R^8 independently; amino; =O; =S; -CO-R^5; -COOR^5, carboxy; cyano; nitro; halogen; hydroxy; -SH; -SR^5; -CONH_2; -CONH(R^5); -CON(R^5)$_2$; C_1-C_{10} alkyloxy optionally substituted with one or more R^8 independently.

In another embodiment of the compounds of formula III, R^4 is C_1-C_{10} alkyl optionally substituted with one or more R^8 independently; C_2-C_{10} alkenyl optionally substituted with one or more R^8 independently; C_2-C_{10} alkynyl optionally substituted with one or more R^8 independently; C_3-C_7 cycloalkyl optionally substituted with one or more R^8 independently; phenyl optionally substituted with one or more R^8 independently; amino; =O; =S; -CO-R^5; -COOR^5, carboxy; cyano; nitro; halogen; hydroxy; -SH; -SR^5; -CONH_2; -CONH(R^5); -CON(R^5)$_2$.

In another embodiment of the compounds of formula III, R^5 is C_1-C_{10} alkyl optionally substituted with one or more R^8 independently; C_2-C_{10} alkenyl optionally substituted with one or more R^8 independently; C_3-C_7 cycloalkyl optionally substituted with one or more R^8 independently; aryl optionally substituted with one or more R^8 independently; aryl C_1-C_6 alkyl optionally substituted with one or more R^8 independently; heteroaryl optionally substituted with one or more R^8 independently; heteroaryl C_1-C_6 alkyl optionally substituted with one or more R^8 independently

In another embodiment of the compounds of formula III, R^6 is H; C_1-C_{10} alkyl optionally substituted with one or more R^4 independently; C_2-C_{10} alkenyl optionally substituted with one or more R^4 independently; C_3-C_7 cycloalkyl optionally substituted with one or more R^4 independently; phenyl optionally substituted with one or more R^4 independently.

In another embodiment of the compounds of formula III, R^7 is H; C_1-C_{10} alkyl optionally substituted with one or more R^4 independently; C_2-C_{10} alkenyl optionally substituted with one or more R^4 independently; C_3-C_7 cycloalkyl optionally substituted with one or more R^4 independently; phenyl optionally substituted with one or more R^4 independently.

In another embodiment of the compounds of formula III, R^8 is H, nitro, tetrazole; -CH_2OH; -CHO; -CF_3; -OCF_3; -CN; -CO_2H; -NH_2; halogen; -OH; -SH; -SCH_3.

In another embodiment of the compounds of formula III, R^9 is H

In another embodiment of the compounds of formula III, R^{10} is H

The following compounds are preferred:

2-(8-(3-Aminopiperidin-1-yl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-7-ylmethyl)benzonitrile

8-(3-Aminopyrrolidin-1-yl)-7-benzyl-1,3-dimethyl-3,7-dihydropurine-2,6-dione

(S) 8-(3-Aminopyrrolidin-1-yl)-7-benzyl-1,3-dimethyl-3,7-dihydropurine-2,6-dione

2-(8-(3-Aminopyrrolidin-1-yl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-7-ylmethyl)benzonitrile

8-(3-Aminopyrrolidin-1-yl)-7-(2-iodobenzyl)-1,3-dimethyl-3,7-dihydropurine-2,6-dione

8-(3-Aminoazepan-1-yl)-7-benzyl-1,3-dimethyl-3,7-dihydropurine-2,6-dione

(S) 8-(3-Aminopyrrolidin-1-yl)-7-(2-iodobenzyl)-1,3-dimethyl-3,7-dihydropurine-2,6-dione

(S) 2-(8-(3-Aminopyrrolidin-1-yl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-7-ylmethyl)benzonitrile

8-(3-Aminopiperidin-1-yl)-7-(2-iodobenzyl)-1,3-dimethyl-3,7-dihydropurine-2,6-dione

8-(3-Aminopiperidin-1-yl)-7-(2-bromobenzyl)-1,3-dimethyl-3,7-dihydropurine-2,6-dione

(R) 8-(3-Aminopyrrolidin-1-yl)-7-(2-bromobenzyl)-1,3-dimethyl-3,7-dihydropurine-2,6-dione

(S) 8-(3-Aminopyrrolidin-1-yl)-7-(2-bromobenzyl)-1,3-dimethyl-3,7-dihydropurine-2,6-dione

(R) 8-(3-Aminopyrrolidin-1-yl)-7-benzyl-1,3-dimethyl-3,7-dihydropurine-2,6-dione

(R) 2-(8-(3-Aminopyrrolidin-1-yl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-7-ylmethyl)benzonitrile

(R) 8-(3-Aminopyrrolidin-1-yl)-7-(2-iodobenzyl)-1,3-dimethyl-3,7-dihydropurine-2,6-dione.

Cis-8-(2-Aminocyclohexylamino)-7-benzyl-3-methyl-1-(2-oxo-2-phenylethyl)-3,7-dihydropurine-2,6-dione

Cis-8-(2-Aminocyclohexylamino)-7-(2-chlorobenzyl)-1-(2-hydroxy-2-phenylethyl)-3-methyl-3,7-dihydropurine-2,6-dione

Trans-8-(2-(S)-Amino-cyclohexyl-(S)-amino)-7-(2-iodo-benzyl)-3-methyl-3,7-dihydro-purine-2,6-dione

Trans-8-(2-(R)-Amino-cyclohexyl-(R)-amino)-7-(2-iodo-benzyl)-3-methyl-3,7-dihydro-purine-2,6-dione

Cis-8-(2-Amino-cyclohexylamino)-7-(2-iodo-benzyl)-3-methyl-3,7-dihydro-purine-2,6-dione

Trans-8-(2-(S)-Amino-cyclohexyl-(S)-amino)-7-biphenyl-2-ylmethyl-3-methyl-3,7-dihydro-purine-2,6-dione

Cis-8-(2-Amino-cyclohexylamino)-7-biphenyl-2-ylmethyl-3-methyl-3,7-dihydro-purine-2,6-dione

Cis-8-(2-(S)-Amino-cyclohexyl-(S)-amino)-7-(2-bromo-benzyl)-3-methyl-3,7-dihydro-purine-2,6-dione

Cis-8-(2-Amino-cyclohexylamino)-7-(2-bromo-benzyl)-3-methyl-3,7-dihydro-purine-2,6-dione

Trans-8-(2-(S)-Amino-cyclohexyl-(S)-amino)-7-(2-chloro-benzyl)-3-methyl-3,7-dihydro-purine-2,6-dione

Trans-8-(2-(R)-Amino-cyclohexyl-(R)-amino)-7-(2-chloro-benzyl)-3-methyl-3,7-dihydro-purine-2,6-dione

Cis-8-(2-Amino-cyclohexylamino)-7-(2-chloro-benzyl)-3-methyl-3,7-dihydro-purine-2,6-dione

Cis-8-(2-Amino-cyclohexylamino)-1,7-bis-(2-chloro-benzyl)-3-methyl-3,7-dihydro-purine-2,6-dione

Cis-2-[8-(2-Amino-cyclohexylamino)-7-(2-chloro-benzyl)-3-methyl-2,6-dioxo-2,3,6,7-tetrahydro-purin-1-ylmethyl]-benzonitrile

Cis-8-(2-Amino-cyclohexylamino)-7-(2-chloro-benzyl)-3-methyl-1-(2-oxo-2-phenyl-ethyl)-3,7-dihydro-purine-2,6-dione

Cis-8-(2-Amino-cyclohexylamino)-7-(2-chloro-benzyl)-3-methyl-1-phenethyl-3,7-dihydro-purine-2,6-dione

Cis-8-(2-Amino-cyclohexylamino)-7-(2-bromo-benzyl)-1-(2-chloro-benzyl)-3-methyl-3,7-dihydro-purine-2,6-dione

Cis-2-[8-(2-Amino-cyclohexylamino)-7-(2-bromo-benzyl)-3-methyl-2,6-dioxo-2,3,6,7-tetrahydro-purin-1-ylmethyl]-benzonitrile

Cis-8-(2-Amino-cyclohexylamino)-7-(2-bromo-benzyl)-3-methyl-1-(2-oxo-2-phenyl-ethyl)-3,7-dihydro-purine-2,6-dione

Cis-8-(2-Amino-cyclohexylamino)-7-(2-bromo-benzyl)-3-methyl-1-phenethyl-3,7-dihydro-purine-2,6-dione

The Neutral Endopeptidase Inhibitor used in a combination treatment according to the invention may be selected from known NEP or dual NEP/ACE inhibitors or prodrugs of such inhibitors. Inhibitors or prodrugs thereof are e.g. known from EP 509442, EP 599444, EP 544620, EP 136883, EP 640594, EP 738711, EP 830863, EP 733642, WO 96/14293, WO 94/15908, WO 93/09101, WO 91/09840, EP 519738, EP 690070, EP 274234, EP 629627, EP 358398, and EP 1097719.

In a preferred embodiment, the NEP inhibitor used in a combination treatment according to the invention is candoxatril, which is a prodrug of candoxatrilat.

In another embodiment the NEP inhibitor used in a combination treatment according to the invention is a dual NEP/ACE inhibitor.

In another embodiment the dual NEP/ACE inhibitor is omapatrilat.

ACE inhibitors may be benazepril, captopril, enalapril, fosinopril, lisinopril, quinapril and ramipril.

The compounds of the present invention may be prepared in the form of pharmaceutically acceptable salts, especially acid-addition salts, including salts of organic acids and mineral acids. Examples of such salts include salts of organic acids such as formic acid, fumaric acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, succinic acid, malic acid, tartaric acid, citric acid, benzoic acid, salicylic acid and the like. Suitable inorganic acid-addition salts include salts of hydrochloric, hydrobromic, sulphuric and phosphoric acids and the like. Further examples of pharmaceutically acceptable inorganic or organic acid addition salts include the pharmaceutically acceptable salts listed in Journal of Pharmaceutical Science, 66, 2 (1977) that are known to the skilled artisan.

Also intended as pharmaceutically acceptable acid addition salts are the hydrates that the present compounds are able to form.

The acid addition salts may be obtained as the direct products of compound synthesis. In the alternative, the free base may be dissolved in a suitable solvent containing the appropriate acid, and the salt isolated by evaporating the solvent or otherwise separating the salt and solvent.

The compounds of this invention may form solvates with standard low molecular weight solvents using methods known to the skilled artisan.

It is to be understood that the invention extends to all of the stereo isomeric forms of the claimed compounds, as well as the racemates.

PHARMACEUTICAL COMPOSITIONS

In another aspect, the present invention includes within its scope pharmaceutical compositions comprising, as active ingredient, both compounds of the invention, i.e. both a DPP-IV inhibitor and a NEP inhibitor, or a pharmaceutically acceptable salt or prodrug or hydrate thereof together with a pharmaceutically acceptable carrier or diluent.

In another aspect, the present invention includes within its scope pharmaceutical compositions comprising, as active ingredient, a compound of the invention having dual inhibitory action, i.e. a compound which inhibits both DPP-IV and NEP.

In another aspect, the present invention includes within its scope pharmaceutical compositions comprising, as active ingredient, one of the inhibitors, where said composition is meant to be used in a regimen where a DPP-IV inhibitor and a NEP inhibitor is to be administered separately. Pharmaceutical compositions containing a compound of the invention of the present invention may be prepared by conventional techniques, e.g. as described in Remington: The Science and Practise of Pharmacy, 19th Ed., 1995. The compositions may appear in conventional forms, for example capsules, tablets, aerosols, solutions, suspensions or topical applications.

Typical compositions include a compound of the invention which inhibits the enzymatic activity of DPP-IV or a pharmaceutically acceptable basic addition salt or prodrug or hydrate thereof, associated with a pharmaceutically acceptable excipient which may be a carrier or a diluent or be diluted by a carrier, or enclosed within a carrier which can be in the form of a capsule, sachet, paper or other container. In making the compositions, conventional techniques for the preparation of pharmaceutical compositions may be used. For example, the active compound will usually be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier which may be in the form of a ampoule, capsule, sachet, paper, or other container. When the carrier serves as a diluent, it may be solid, semi-solid, or liquid material which acts as a vehicle, excipient, or medium for the active compound. The active compound can be adsorbed on a granular solid container for example in a sachet. Some examples of suitable carriers are water, salt solutions, alcohols, polyethylene glycols, polyhydroxyethoxylated castor oil, peanut oil, olive oil, gelatine, lactose, terra alba, sucrose, dextrin, magnesium carbonate, sugar, cyclodextrin, amylose, magnesium stearate, talc, gelatine, agar, pectin, acacia, stearic acid or lower alkyl ethers of cellulose, silicic acid, fatty acids, fatty acid amines, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, polyoxyethylene, hydroxymethylcellulose and polyvinylpyrrolidone. Similarly, the carrier or diluent may include any sustained release material known in the art, such as glyceryl monostearate or glyceryl distearate, alone or mixed with a wax. The formulations may also include wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavouring agents. The formulations of the invention may be formulated so as to provide quick, sustained, or delayed release of the active ingredient after administration to the patient by employing procedures well known in the art.

The pharmaceutical compositions can be sterilized and mixed, if desired, with auxiliary agents, emulsifiers, salt for influencing osmotic pressure, buffers and/or colouring substances and the like, which do not deleteriously react with the active compounds.

The route of administration may be any route, which effectively transports the active compound of the invention which inhibits the enzymatic activity of DPP-IV to the appropriate or desired site of action, such as oral, nasal, pulmonary, buccal, subdermal, intradermial, transdermal or parenteral e.g. rectal, depot, subcutaneous, intravenous, intraurethral, intramuscular, intranasal, ophthalmic solution or an ointment, the oral route being preferred.

If a solid carrier is used for oral administration, the preparation may be tabletted, placed in a hard gelatin capsule in powder or pellet form or it can be in the form of a troche or lozenge. If a liquid carrier is used, the preparation may be in the form of a syrup, emulsion, soft gelatin capsule or sterile injectable liquid such as an aqueous or non-aqueous liquid suspension or solution.

For nasal administration, the preparation may contain a compound of the invention which inhibits the enzymatic activity of DPP-IV, dissolved or suspended in a liquid carrier, in particular an aqueous carrier, for aerosol application. The carrier may contain additives such as solubilizing agents, e.g. propylene glycol, surfactants, absorption enhancers such as lecithin (phosphatidylcholine) or cyclodextrin, or preservatives such as parabenes.

For parenteral application, particularly suitable are injectable solutions or suspensions, preferably aqueous solutions with the active compound dissolved in polyhydroxylated castor oil.

Tablets, dragees, or capsules having talc and/or a carbohydrate carrier or binder or the like are particularly suitable for oral application. Preferable carriers for tablets, dragees, or capsules include lactose, corn starch, and/or potato starch. A syrup or elixir can be used in cases where a sweetened vehicle can be employed.

A typical tablet which may be prepared by conventional tabletting techniques may contain:

Core:

Active compound (as free compound or salt thereof)	250 mg
Colloidal silicon dioxide (Aerosil)®	1.5 mg
Cellulose, microcryst. (Avicel)®	70 mg
Modified cellulose gum (Ac-Di-Sol)®	7.5 mg
Magnesium stearate	**Ad.**

Coating:

HPMC approx.	9 mg
*Mywacett 9-40 T approx.	0.9 mg

*Acylated monoglyceride used as plasticizer for film coating.

The compounds of the invention are effective over a wide dosage range. For example, in the treatment of adult humans, dosages from about 0.05 to about 1000 mg, preferably from about 0.1 to about 500 mg, per day may be used. A most preferable dosage is about 0.5 mg to about 250 mg per day. In choosing a regimen for patients it may frequently be necessary to begin with a higher dosage and when the condition is under control to reduce the dosage. The exact dosage will depend upon the mode of administration, on the therapy desired, form in which administered, the subject to be treated and the body weight of the subject to be treated, and the preference and experience of the physician or veterinarian in charge.
Generally, the compounds of the present invention are dispensed in unit dosage form comprising from about 0.05 to about 1000 mg of active ingredient together with a pharmaceutically acceptable carrier per unit dosage.
Usually, dosage forms suitable for oral, nasal, pulmonal or transdermal administration comprise from about 0.05 mg to about 1000 mg, preferably from about 0.5 mg to about 250 mg of the compounds admixed with a pharmaceutically acceptable carrier or diluent.
The invention also encompasses prodrugs of a compound of the invention which on administration undergo chemical conversion by metabolic processes before becoming active pharmacological substances. In general, such prodrugs will be functional derivatives of a compound af the invention which are readily convertible in vivo into a compound af the invention.
Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in "Design of Prodrugs", ed. H. Bundgaard, Elsevier, 1985.

EXAMPLES

Preparative HPLC (Method A1)

Column: 1.9 x 15 cm Waters XTerra RP-18. Buffer: linear gradient 5 – 95% in 15 min, MeCN, 0.1% TFA, flow rate of 15 ml/min. The pooled fractions are either evaporated to dryness *in vacuo*, or evaporated *in vacuo* until the MeCN is removed, and then frozen and freeze dried.

Preparative HPLC (Method A2)

Column: Supelcosil ABZ+Plus, 25 cm x 10 mm, 5 µm. Solvent A: 0.1% TFA/Water, solvent B: MeCN. Eluent composition: 5 min. 100% A, linear gradient 0 – 100% B in 7 min, 100% B in 2 min. Flow rate 5 ml/min. The column is allowed to equilibrate for 4 min in 100% A before the next run.

HPLC-MS (Method B)

Column: Waters Xterra MS C-18 X 3 mm id. Buffer: Linear gradient 10 - 100% in 7.5 min, MeCN, 0.01% TFA, flow rate 1.0 ml/min. Detection 210 nm (analog output from diode array detector), MS-detection ionisation mode API-ES, scan 100-1000 amu step 0.1 amu.

HPLC-MS (Method C)

Column: 0.3 mm x 15 cm Waters Symmetry C_{18}. Buffer: Linear gradient 5 - 90% in 15 min, MeCN, 0.05% TFA, flow rate 1 ml/min

Analytical separation of stereoisomers (Method D)

CCE. Chiral capillary electrophoresis: Conditions: HP 3D Capillary Electrophoresis: 48.5/40cm, 50µm HP bubble capillary, Electrolyte: HS-β-CD (Regis) (2% w/v) in 50mM phosphate buffer pH2.5 (HP), Voltage: -17kV, Injection: 30mbar for 5s.

Preparative separation of stereoisomers (Method E)

Analytical separations were performed on Hewlett Packard 1090 HPLC equipment with 5 chiral Daicel columns (AD, OD, AS, OJ and Welko-O2, 250 x 4.6 mm) with a diode array detector. The mobile phases were 2-propanol:heptane mixtures with 0.1% DEA.

Preparative separations were performed with the above-mentioned type of columns (250 x 20 mm) on a preparative Gilson HPLC set-up. Relevant fractions were collected and evaporated (SpeedVac).

Microwave assisted reactions (Method F)

The reactants are mixed in an appropriate solvent in a closed teflon vessel (XP 1500 Plus Vessel set) and heated in a micro wave oven (CEM MARSX microwave instrument. Magnetron frequency: 2455 MHz. Power Output: 1200 Watt.). The reaction mixture is cooled and evaporated *in vacuo*. Normally solvents like MeOH; EtOH, iPrOH; H2O; DMF and DMSO are used.

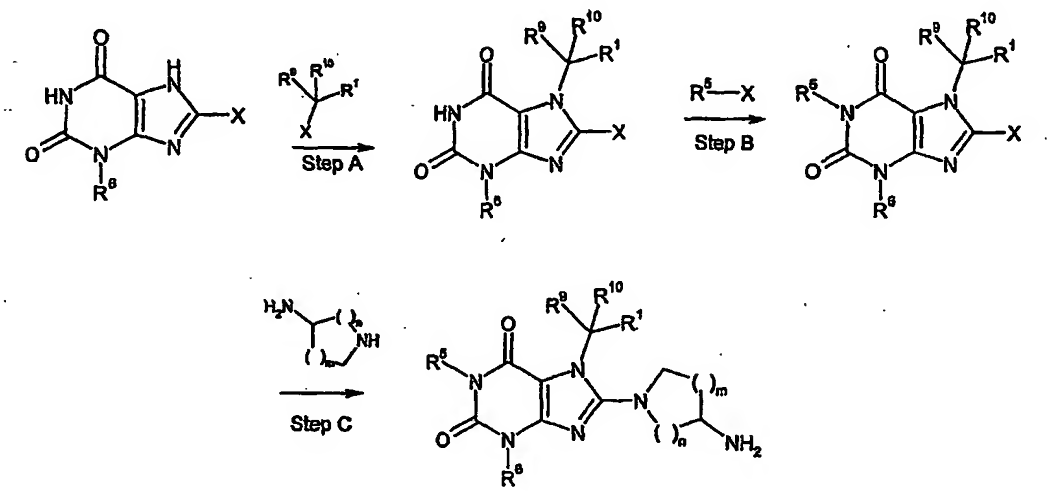
Abbreviations

DCHMA	Dicyclohexylmethylamine

EtOAc	Ethyl acetate
DCM	Dichloromethane
DEA	Diethylamine
DIEA	Diisopropylethylamine
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
HOAc	Acetic acid
MeCN	Acetonitrile
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TMG	Tetramethylguanidine

The compounds of Formula I are prepared according to the following procedures:

General procedure (A):



Step A:

The starting material (16 µmol) is dissolved in a mixture of DMF and DIEA (3% DIEA, 250 µl). The alkylation reagent R^1-CR^9R^9-X (16.8 µmol, 1.05 equiv) is dissolved in DMF (100 µl) and added. The mixture is heated to 65 °C for 2h.

Step B:

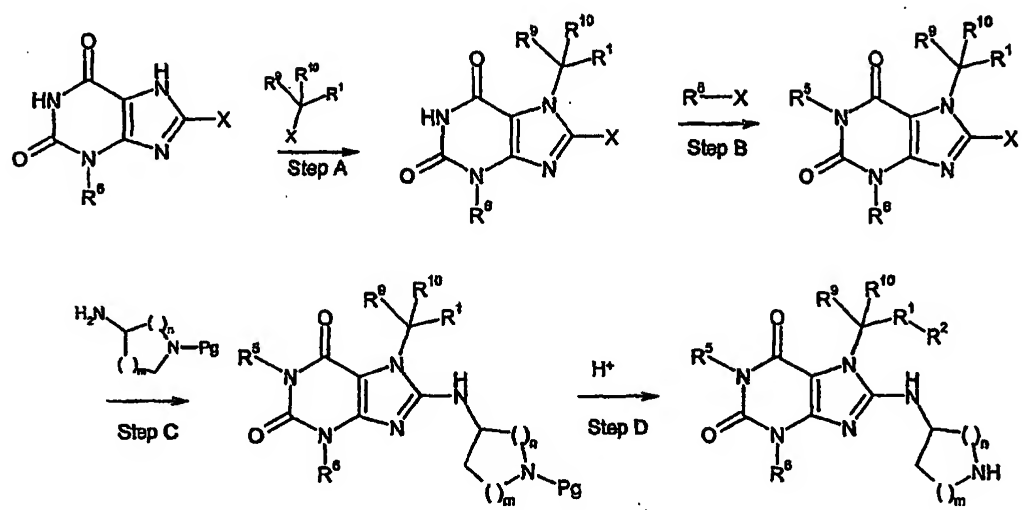
Alkylation reagent R^5-Br (32 µmol) is dissolved in DMF (100 µl) and added to the reaction mixture followed by a solution of TMG in DMF (1.16 ml TMG diluted to 5.8 ml, 48 µl). The mixture is kept at 65 °C for 4h.

Step C:

The diamine (200 µmol) is dissolved in a mixture of DMF and DIEA (3% DIEA, 200 µl) and added to the reaction mixture. The reaction is kept at 50 °C for 24h.

Samples are neutralized using HOAc (20 µl), stripped and purified by HPLC. Samples are dissolved in DMSO/H_2O (4:1, 500 µl).

General procedure (B)



Step A:

The starting material (16 µmol) is dissolved in a mixture of DMF and DIEA (3% DIEA, 250 µl). The alkylation reagent R^1-CR^9R^9-X (16.8 µmol, 1.05 equiv) is dissolved in DMF (100 µl) and added. The mixture is heated to 65°C for 2h.

Step B:

Alkylation reagent R^5-Br (32 µmol) is dissolved in DMF (100 µl) and added to the reaction mixture followed by a solution of TMG in DMF (1.16 ml TMG diluted to 5.8 ml, 48 µl). The mixture is kept at 65°C for 4h.

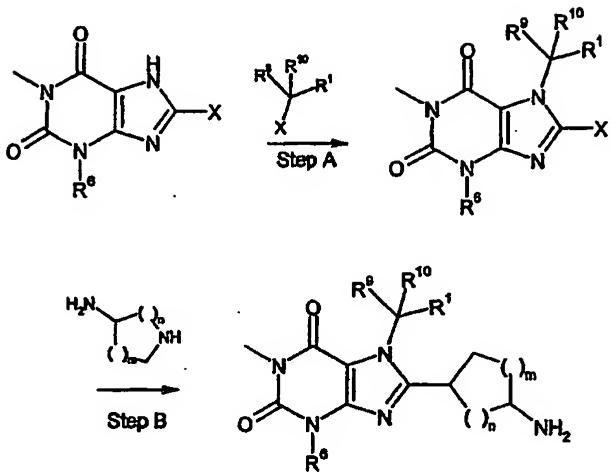
Step C:

The monoprotected diamine (200 µmol) is dissolved in a mixture of DMF and DIEA (3% DIEA, 200 µl) and added to the reaction mixture. The reaction is kept at 50°C for 24-48h, and then all volatiles are stripped.

Step D:

TFA (2 ml) is added, and the reaction is kept for 16 h at room temperature. The reactions are stripped from excess TFA, taken up in acetonitrile, and purified by HPLC (method A). Samples are dissolved in DMSO/H_2O (4:1, 500 µl).

General procedure (C)



Step A:

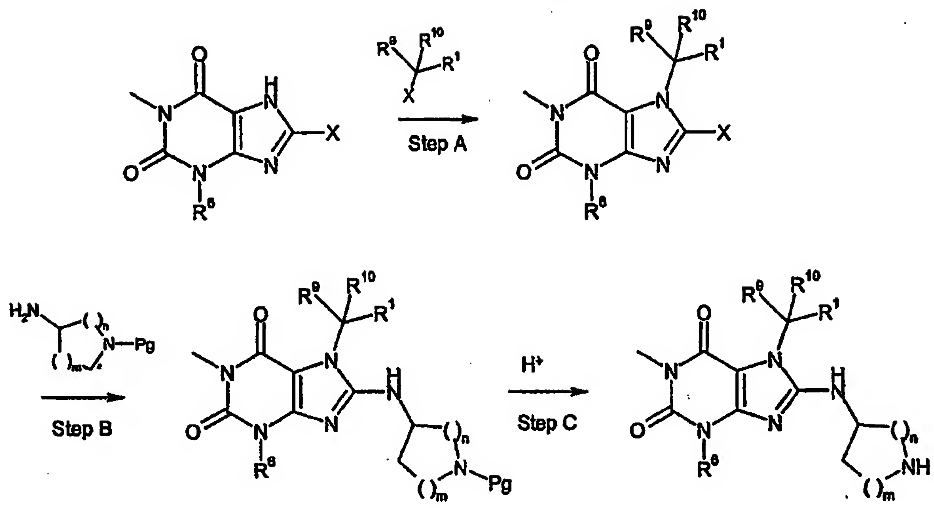
The starting material (16 μmol) is dissolved in a mixture of DMF and DIEA (3% DIEA, 250 μl). The alkylation reagent R^1-CR^9R^9-X (16.8 μmol, 1.05 equiv) is dissolved in DMF (100 μl) and added. The mixture is heated to 65°C for 2h.

Step B:

Diamine (200 μmol) is dissolved in a mixture of DMF and DIEA (3% DIEA, 200 μl) and added to the reaction mixture. The reaction is kept at 50°C for 24-48h, and then all volatiles are stripped.

Samples are neutralized using HOAc (20 μl), stripped and purified by HPLC. Samples are dissolved in DMSO/H_2O (4:1, 500 μl).

General procedure (D)



Step A:

The starting material (16 µmol) is dissolved in a mixture of DMF and DIEA (3% DIEA, 250 µl). The alkylation reagent R^1-CR^9R^9-X (16.8 µmol, 1.05 equiv) is dissolved in DMF (100 µl) and added. The mixture is heated to 65°C for 2h..

Step B:

The monoprotected diamine (200 µmol) is dissolved in a mixture of DMF and DIEA (3% DIEA, 200 µl) and added to the reaction mixture. The reaction is kept at 50°C for 24-48h, and then all volatiles are stripped.

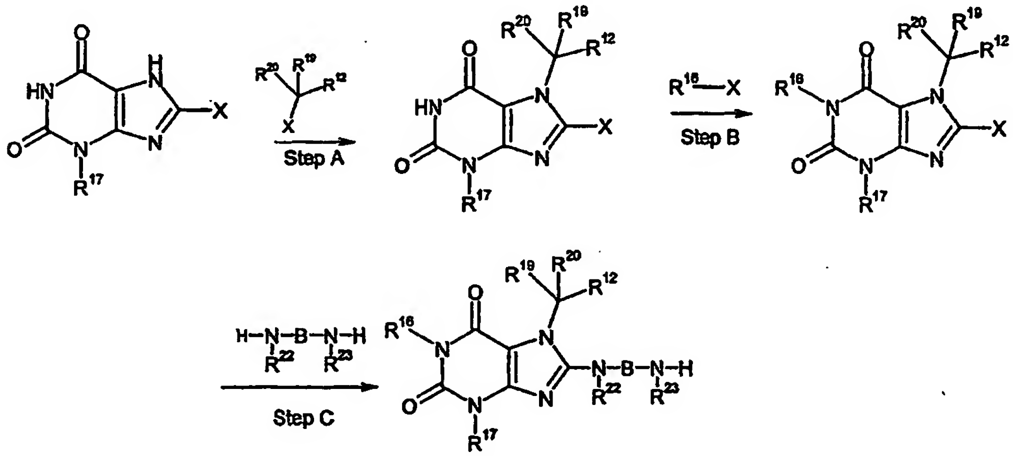
Step C:

TFA (2 ml) is added, and the reaction is kept for 16 h at room temperature. The reactions are stripped from excess TFA, taken up in acetonitrile, and purified by HPLC (method A). Samples are dissolved in DMSO/H_2O (4:1, 500 µl).

The R-groups in the general methods above are as defined in the description of the invention section. The Pg group is an acid labile N-protection group such as Boc or trityl.

The compounds of Formula II are prepared according to the following procedure:

General procedure (E):



Step A:

The starting material (16 µmol) is dissolved in a mixture of DMF and DIEA (3% DIEA, 250 µl). The alkylation reagent R^{12}-$CR^{19}R^{20}$-X (16.8 µmol, 1.05 equiv) is dissolved in DMF (100 µl) and added. The mixture is heated to 65 °C for 2h.

Step B:

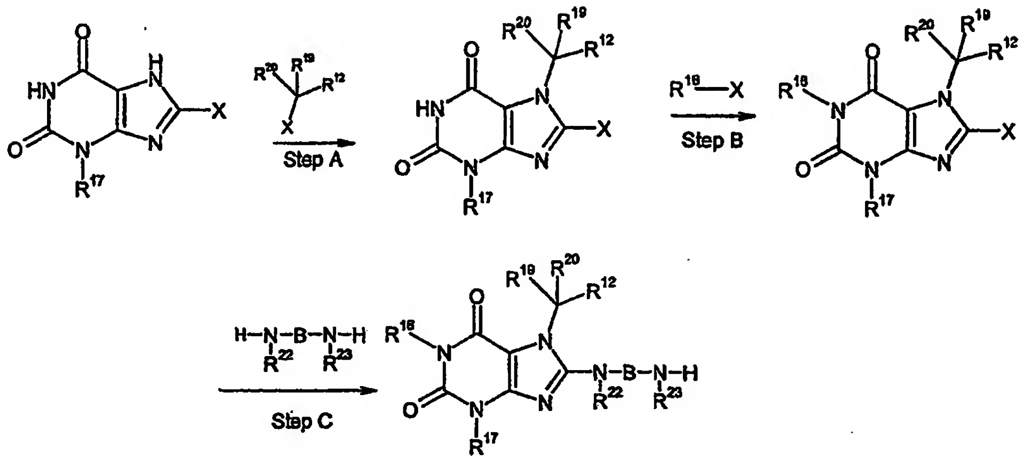
Alkylation reagent R^{16}-Br (32 µmol) is dissolved in DMF (100 µl) and added to the reaction mixture followed by a solution of TMG in DMF (1.16 ml TMG diluted to 5.8 ml, 48 µl). The mixture is kept at 65 °C for 4h. Volatiles are stripped

Step C:

The diamine (200 µmol) is dissolved in a mixture of DMSO and DCHMA (3% DCHMA, 200 µl) and added to the reaction mixture. The reaction is kept at 50 °C for 44h.

Samples are neutralized using HOAc (20 µl), stripped and purified by HPLC Method A2..

General procedure (F):



Step A:

The starting material (32 µmol) is dissolved in a mixture of DMF and DIEA (3% DIEA, 500 µl). The alkylation reagent R^{12}-$CR^{19}R^{20}$-X (33.6 µmol, 1.05 equiv) is dissolved in DMF (200 µl) and added. The mixture is heated to 65 °C for 2h. Upon cooling to 25 °C, K_2CO_3 (aq) is added (5.12M, 50 µL, 256 umol). Volatiles are stripped.

Step B:

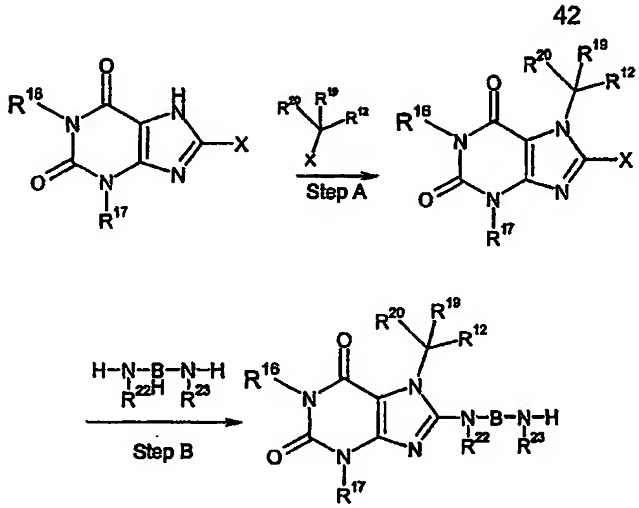
Alkylation reagent R^{16}-Br (64 µmol) is dissolved in DMF (250 µl) and added to the reaction mixture. The mixture is kept at 25 °C for 48h. Volatiles are stripped

Step C:

The diamine (400 µmol) is dissolved in DMSO and added to the reaction mixture. If the dihydrochloride salt of the diamine is employed, four equivalents of DCHMA is added. The reaction is kept at 50 °C for 48h.

Samples are neutralized using HOAc (30 µl), and purified by HPLC Method (HDEM).

General procedure (G)



Step A:

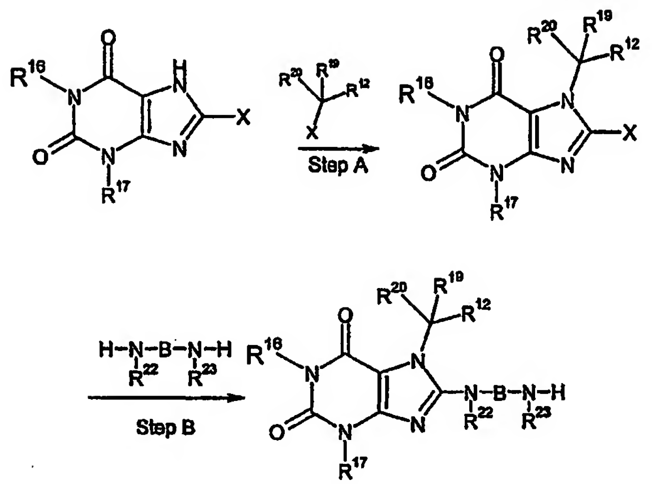
The starting material (4.08 mmol) is dissolved in a mixture of DMF and DIEA (3% DIEA, 65 ml). The alkylation reagent R^{12}-$CR^{19}R^{20}$-X (4.28 mmol, 1.05 equiv) is dissolved in DMF (25.5 ml) and added. The mixture is heated to 65°C for 2h and poured onto ice followed by filtration of the alkylated product.

Step B:

Diamine (400 µmol) is dissolved in DMSO (400 µl) and added to the above product (32 umol). The reaction is kept at 50°C for 24-48h.

Samples are neutralized using HOAc (30 µl) and purified by HPLC Method A2.

General procedure (H)



Step A:

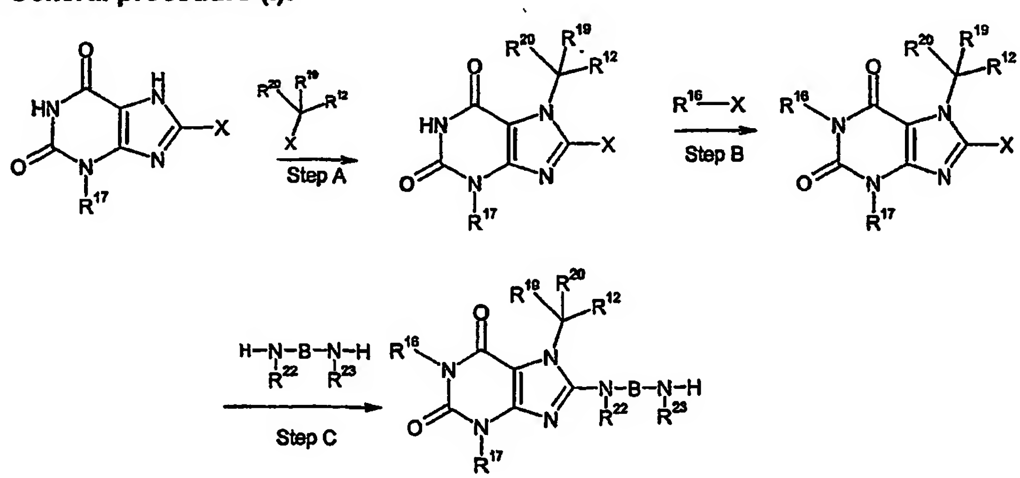
The starting material (32 µmol) is dissolved in a mixture of DMF and DIEA (3% DIEA, 500 µl). The alkylation reagent R^{12}-$CR^{19}R^{20}$-X (33.6 µmol, 1.05 equiv) is dissolved in DMF (200 µl) and added. The mixture is heated to 65°C for 2h.

Step B:

Diamine (400 µmol) is dissolved in DMSO (400 µl) and added to the above reaction mixture. The reaction is kept at 50°C for 48h.

Samples are neutralized using HOAc (30 µl) and purified by HPLC Method A2.

General procedure (I):



Step A:

The starting material (20.40 mmol) is dissolved in DMF (50 ml) and DIEA (10 mL). The alkylation reagent R^{12}-$CR^{20}R^{19}$-X (22.03 mmol, 1.08 equiv) is dissolved in DMF (10 ml) and added. Heating the mixture to 65 °C for 2h affords the products that are isolated by filtration upon adding the reaction mixture onto ice (300 mL).

Step B:

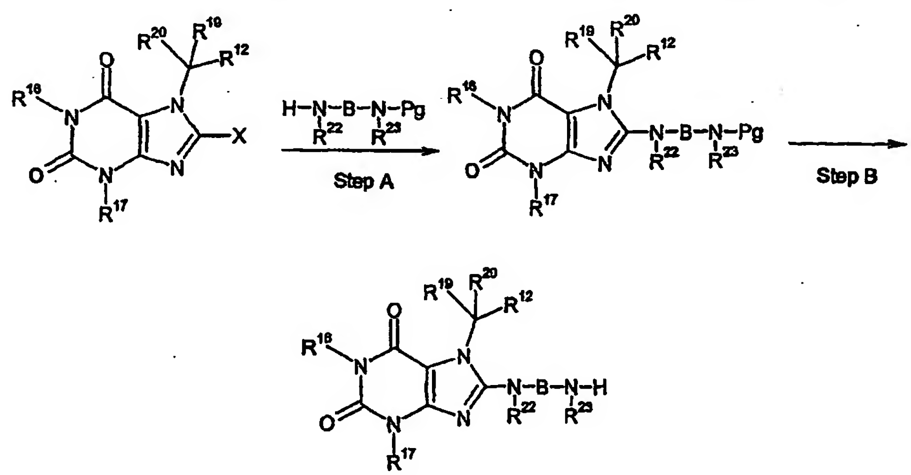
The product from Step A (5.56 mmol) and alkylation reagent R^{16}-Br (11.11 µmol) are dissolved in DMF (60 mL) and potassium carbonate is added to the reaction mixture. Upon stirring at 25 °C for 16h the reaction mixture is poured onto ice (300 ml) and the product is isolated by filtration and dried *in vacuo*.

Step C:

The product from Step B (0.472 mmol) is dissolved in DMSO (5 ml) and the diamine (2.36 mmol) is added to the reaction mixture. If the dihydrochloride salt of the diamine is employed, K_2CO_3 (2.36 mmol) is added. The reaction is kept at 50 °C for 24h and poured onto ice (20 ml). The product is isolated by filtration.

General procedure (J) for removal of protection groups (Pg):

Sometimes mono-protected diamines are employed in the final substitution reaction. In these cases, an extra synthesis step is required to remove the protection group.



Step A

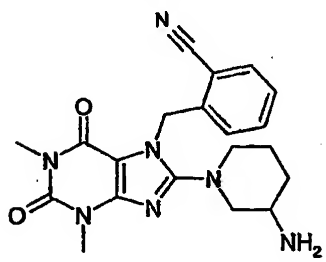
The product generated in Step A may be obtained *via* any of the above-mentioned general procedures.

Step B

The conditions required depend on the nature of the protecting group.

Example 1

2-(8-(3-Aminopiperidin-1-yl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-7-ylmethyl)benzonitrile. TFA



Step A: 2-(8-Chloro-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-7-ylmethyl)benzonitrile (1A)

8-Chlorotheophylline (20 g, 93.19 mmol) was dissolved in 800 ml of DMF and 2-cyanobenzyl bromide (18.28 g, 93.19 mmol), potassium carbonate (12.88 g, 93.19 mmol), and potassium iodide (10 mg, 0.06 mmol) were added. The mixture was stirred at room temperature for 20 hours. The solvent was evaporated and the residue was suspended in 900 ml of water and 900 ml of EtOAc, and compound (1A) was collected by filtration of the suspension. The layers in the mother liquor were separated and the aqueous layer was extracted with 3 x 500 ml of EtOAc. The combined organic layers were washed with 1 x 500 ml of water, and the solvent was evaporated to give compound (1A) as white crystals.

Combined yield: 28.6 g (93%). Mp. 222.5 - 223.7°C.

^{1}H-NMR (DMSO, 300 MHz) δ: 3.20 (s, 3H); 3.43 (s, 3H); 5.74 (s, 2H); 7.06 (d, 1H); 7.53 (t, 1H); 7.67 (t, 1H); 7.93 (d, 1H). HPLC-MS (Method B): *m/z* = 330 (M+1); R_t = 2.93 min.

Step B: 2-(8-(3-Aminopiperidin-1-yl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-7-ylmethyl)benzonitrile. TFA (1)

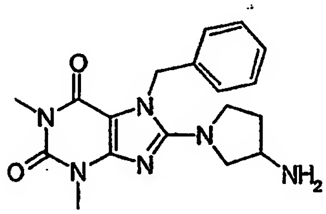
2-(8-Chloro-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-7-ylmethyl)benzonitrile (1A) (100 mg, 0.30 mmol) and 3-aminopiperidine dihydrochloride (262 mg, 1.52 mmol) were dissolved in 20 ml of 2-propanol and triethylamine (0.127 ml, 0.91 mmol) and subjected to microwaves (method F, 130°C, 300W) for ten hours. The solvents were evaporated and the crude product was purified by preparative HPLC, (method A1, Rt = 6.78 min.) to give the title compound as oily crystals.

Yield: 66 mg (43%).

^{1}H-NMR (MeOD, 300 MHz) δ: 1.73 (m, 3H); 2.10 (m, 1H); 3.02 (m, 1H); 3.20 (m, 2H); 3.27 (s, 3H); 3.52 (m, 4H); 3.65 (m, 1H); 5.59 (s, 2H); 7.22 (d, 1H); 7.47 (m, 1H); 7.61 (m, 1H); 7.78 (d, 1H). HPLC-MS (Method B): m/z = 394 (M+1); R_t = 1.55 min.

Example 2

8-(3-Aminopyrrolidin-1-yl)-7-benzyl-1,3-dimethyl-3,7-dihydropurine-2,6-dione. HCl



Step A: 7-Benzyl-8-chloro-1,3-dimethyl-3,7-dihydropurine-2,6-dione (2A)

8-Chlorotheophylline (50 g, 0.23 mol) was suspended in 600 ml of DMF and benzyl bromide (31 ml, 0.26 mol) and potassium carbonate (64 g, 0.46 mol) were added. The mixture was stirred at room temperature for 20 hours. The solvent was evaporated and the residue was dissolved in 250 ml of water and 400 ml of DCM. The layers were separated and the aqueous layer was extracted with 150 ml of DCM. The combined organic layer was washed with 100 ml of brine, dried over magnesium sulphate, filtered, and the solvent was evaporated to give compound (2A) as white crystals.

Yield: 73.6 g (104%). Mp. 152 - 154°C.

^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 3.42 (s, 3H); 3.55 (s, 3H); 5.55 (s, 2H); 7.35 (m, 5H). HPLC-MS (Method B): m/z = 305 (M+1); R_t = 3.33 min.

Step B: 8-(3-Aminopyrrolidin-1-yl)-7-benzyl-1,3-dimethyl-3,7-dihydropurine-2,6-dione. HCl (2)

7-Benzyl-8-chloro-1,3-dimethyl-3,7-dihydropurine-2,6-dione (2A) (100 mg, 0.33 mmol) and 3-aminopyrrolidine (0.16 ml, 1.64 mmol) were dissolved in 20 ml of 2-propanol and subjected to microwaves (method F, 150°C, 300W) for one hour. The solvent was evaporated and the crude product was purified by preparative HPLC (method A1, Rt = 6.45 min.). Evaporation of the solvent afforded the title compound as a brown oil.

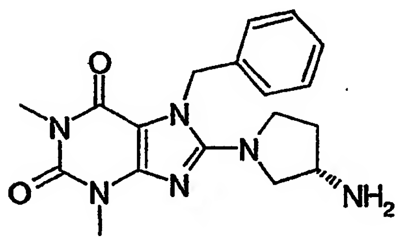
Yield: 111 mg (87%).

^{1}H-NMR (MeOD, 400 MHz) δ: 2.04 (m, 1H); 2.37 (m, 1H); 3.30 (s, 3H); 3.51 (s, 3H); 3.60 - 3.80 (m, 3H); 3.87 - 3.95 (m, 2H); 5.54 (d, 1H); 5.64 (d, 1H); 7.14 (d, 2H); 7.23 - 7.35 (m, 3H)

HPLC-MS (Method B): m/z = 355 (M+1); R_t = 1.49 min.

Example 3

(S) 8-(3-Aminopyrrolidin-1-yl)-7-benzyl-1,3-dimethyl-3,7-dihydropurine-2,6-dione. HCl



Step A: (S) (1-(7-Benzyl-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-8-yl)pyrrolidin-3-yl)carbamic acid tert-butyl ester (3A)

7-Benzyl-8-chloro-1,3-dimethyl-3,7-dihydropurine-2,6-dione (2A) (100 mg, 0.33 mmol), (3S)-(-)-3-(tert-butoxycarbonylamino)pyrrolidine (305 mg, 1.64 mmol), and triethylamine (0.46 ml, 3.28 mmol) was dissolved in 20 ml of 2-propanol and 5 ml of DMF and the mixture was subjected to microwaves (method F, 130°C, 300W) for three hours. The solvent was evaporated and the crude product was purified by preparative HPLC (method A1, Rt = 11.75 min.). Evaporation of the solvent afforded compound (3A) as a brown oil.

Yield: 130 mg (87%)

^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 1.42 (s, 9H); 1.89 (m, 1H); 2.12 (m, 1H); 3.34 (s, 3H); 3.37 - 3.79 (m, 7H); 4.22 (br. s, 1H); 4.97 (d, 1H); 5.49 (d, 1H); 5.55 (d, 1H); 7.04 (m, 2H); 7.28 (m, 3H). HPLC-MS (Method B): m/z = 455 (M+1); R_t = 3.95 min.

Step B: (S) 8-(3-Aminopyrrolidin-1-yl)-7-benzyl-1,3-dimethyl-3,7-dihydropurine-2,6-dione. HCl (3)

(S) (1-(7-Benzyl-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-8-yl)pyrrolidin-3-yl)carbamic acid tert-butyl ester (3A) (130 mg, 0.29 mmol) was dissolved in 15 ml of diethyl ether, hydrochloric acid in diethyl ether (2.5 M, 5.72 ml, 14.3 mmol) was added, and the mixture was stirred at room temperature for 24 hours. The solvents were evaporated and the crude product was suspended in dry DCM and collected by filtration to afford the title compound as white crystals.

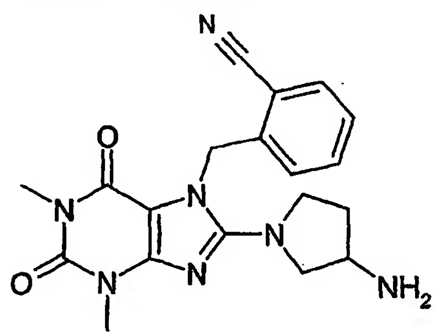
Yield: 101 mg, (91%) Mp. 166 - 169°C.

^{1}H-NMR (MeOD, 300 MHz) δ: 2.05 (m, 1H); 2.37 (m, 1H); 3.29 (s, 3H); 3.52 (s, 3H); 3.58 - 3.97 (m, 5H); 5.53 (d, 1H); 5.63 (d, 1H); 7.13 (d, 2H); 7.21 - 7.36 (m, 3H).

HPLC-MS (Method B): *m/z* = 355 (M+1); R_t = 1.52 min.

Example 4

2-(8-(3-Aminopyrrolidin-1-yl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-7-ylmethyl)benzonitrile. HCl



2-(8-Chloro-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-7-ylmethyl)benzonitrile (1A) (100 mg, 0.30 mmol) and 3-aminopyrrolidine (0.15 ml, 1.52 mmol) were reacted and purified as described in example 2, step B, to give the title compound as a yellow foam.

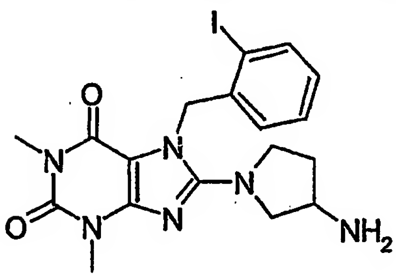
Yield: 108 mg (76%). Mp.186 - 189°C.

Prep. HPLC (method A1): R_t = 6.19 min.

^{1}H-NMR (MeOD, 400 MHz) δ: 2.09 (m, 1H); 2.40 (m, 1H); 3.27 (s, 3H); 3.50 (s, 3H); 3.59 - 3.78 (m, 3H); 3.88 - 3.99 (m, 2H); 5.70 (d, 1H); 5.79 (d, 1H); 7.12 (d, 1H); 7.49 (dd, 1H); 7.62 (dd, 1H); 7.80 (d, 1H). HPLC-MS (Method B): *m/z* = 380 (M+1); R_t = 1.35 min.

Example 5

8-(3-Aminopyrrolidin-1-yl)-7-(2-iodobenzyl)-1,3-dimethyl-3,7-dihydropurine-2,6-dione. HCl



<u>Step A: 8-Chloro-7-(2-iodobenzyl)-1,3-dimethyl-3,7-dihydropurine-2,6-dione (5A)</u>

8-Chlorotheophylline (8.5 g, 39.6 mmol) was dissolved in 400 ml of DMF and 2-iodobenzyl chloride (10.0 g, 39.6 mmol), potassium carbonate (5.47 g, 39.6 mmol), and potassium iodide (10 mg, 0.06 mmol) were added. The mixture was stirred at room temperature for 7 days. Water (2500 ml) and EtOAc (800 ml) were added and the layers were separated. The aqueous layer was extracted with 2 x 500 ml of EtOAc, and the combined organic layer was washed with 500 ml of water, 500 ml of brine, dried over sodium sulphate, and filtered. The solvent was evaporated and the crude product was crystallized from diethyl ether and petrol, to give compound (5A) as white crystals. The mother liquor was evaporated and resuspended in diethyl ether and petrol, to give a second crop of compound (5A).

Combined yield: 10.4 g (61%). Mp. 177.6 - 178.2°C.

^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 3.37 (s, 3H); 3.61 (s, 3H); 5.59 (s, 2H); 6.48 (d, 1H); 7.02 (t, 1H); 7.27 (t, 1H); 7.90 (d, 1H). HPLC-MS (Method B): *m/z* = 431 (M+1); R_t = 3.94 min.

<u>Step B: 8-(3-Aminopyrrolidin-1-yl)-7-(2-iodobenzyl)-1,3-dimethyl-3,7-dihydropurine-2,6-dione. HCl (5)</u>

8-Chloro-7-(2-iodobenzyl)-1,3-dimethyl-3,7-dihydropurine-2,6-dione (5A) (100 mg, 0.23 mmol) and 3-aminopyrrolidine (0.13 ml, 1.16 mmol) were reacted and purified as described in example **2**, <u>step B</u>, to give the crude product, which was further suspended in dry DCM, and filtered to afford the <u>title compound</u> as white crystals.

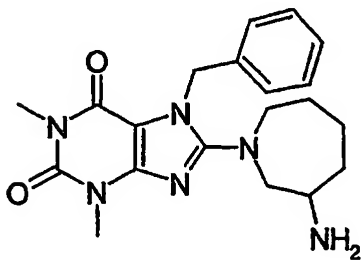
Yield: 77 mg (64%).

Prep. HPLC (method A1): R_t = 7.28 min.

^{1}H-NMR (MeOD, 200 MHz) δ: 2.02 (m, 1H); 2.35 (m, 1H); 3.27 (s, 3H); 3.47 - 3.74 (m, 6H); 3.82 - 3.93 (m, 2H); 5.44 (d, 1H); 5.53 (d, 1H); 6.72 (d, 1H); 7.04 (dd, 1H); 7.32 (dd, 1H); 7.92 (d, 1H). HPLC-MS (Method B): *m/z* = 481 (M+1); R_t = 1.76 min.

Example 6

8-(3-Aminoazepan-1-yl)-7-benzyl-1,3-dimethyl-3,7-dihydropurine-2,6-dione. TFA



Step A: N-(2-Oxoazepan-3-yl)-4-methylbenzenesulfonamide (6A)

DL-3-Amino-E-caprolactam (3 g, 23.4 mmol) was dissolved in 140 ml of dry DCM and dry triethylamine (4.5 ml) and 4-toluenesulfonyl chloride (4.5 g, 23.6 mmol) were added. The reaction was stirred for 3 days at room temperature and then filtered through celite. The filtrate was extracted with 50 ml of 1M aqueous potassium hydrogen sulphate, 50 ml of saturated sodium hydrogen carbonate, 50 ml of water, and 50 ml of brine, and dried over sodium sulphate. The solvent was evaporated and the residue suspended in dry dichloromethane, and compound (6A) was collected by filtration. The mother liquor was evaporated and resuspended in DCM, to give a second crop of compound (6A) as white crystals.

Combined yield: 5.99 g (90%). Mp. 179.9 - 180.5°C.

^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.34 (m, 1H); 1.55 - 1.85 (m, 3H); 2.00 (m, 1H); 2.17 (m, 1H); 2.40 (s, 3H); 3.10 (m, 2H); 3.81 (m, 1H); 5.86 (m, 1H); 6.12 (d, 1H); 7.28 (d, 2H); 7.72 (d, 2H). HPLC-MS (Method B): m/z = 283 (M+1); R_t = 2.71 min.

Step B: N-(Azepan-3-yl)-4-methylbenzenesulfonamide (6B)

N-(2-Oxoazepan-3-yl)-4-methylbenzenesulfonamide (6A) (4.24 g, 15 mmol) was dissolved in 250 ml of dry THF under a nitrogen atmosphere, and lithium aluminium hydride (1.11 g, 30 mmol) was added slowly. The reaction was heated to reflux for 20 hours and then quenched with water until the effervescence ceased. Solid potassium carbonate was added until a white suspension appeared, and the mixture was allowed to stir for half an hour. The suspension was filtered through celite, which was washed with 3 x 50 ml of EtOAc. The solvents were evaporated and the residue was dissolved in 100 ml of EtOAc and 100 ml of water. The layers were separated and the aqueous layer was extracted with 2 x 100 ml of EtOAc. The

combined organic layer was washed with brine, dried over sodium sulphate, and evaporated to give compound (6B) as an oil.

Yield: 2.89 g (71%).

^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.37 - 1.74 (m, 6H); 2.41 (s, 3H); 2.55 - 2.93 (m, 4H); 3.45 (m, 1H); 7.27 (d, 2H); 7.76 (d, 2H). HPLC-MS (Method B): *m/z* = 269 (M+1); R_t = 1.43 min.

<u>Step C: N-(1-(7-Benzyl-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-8-yl)azepan-3-yl)-4-methylbenzenesulfonamide (6C)</u>

7-Benzyl-8-chloro-1,3-dimethyl-3,7-dihydropurine-2,6-dione (2A) (1.03 g, 3.40 mmol) and N-(azepan-3-yl)-4-methylbenzenesulfonamide (6B) (1.00 g, 3.73 mmol) were dissolved in 2-methoxyethanol (30 ml) and triethylamine (2.4 ml), and the mixture was heated to 120°C for 2 days. The solvents were evaporated and the crude product was dissolved in 100 ml of EtOAc and 100 ml of water. The aqueous phase was acidified with 1M potassium hydrogen sulphate until pH = 2. The organic layer was separated and extracted with 50 ml of 1M aqueous potassium hydrogen sulphate, and 50 ml of brine, and dried over sodium sulphate. The solvent was evaporated and the crude product was purified by column chromatography on silica gel using EtOAc:heptane (1:1) as the eluent. Evaporation of the solvent gave compound (6C) as a white foam.

Yield: 548 mg (30%). Mp. 80.2 - 88.2°C.

^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.22 - 1.84 (m, 6H); 2.41 (s, 3H); 3.00 (m, 1H); 3.25 (dd, 1H); 3.47 - 3.72 (m, 6H); 5.37 (d, 1H); 5.59 (d, 1H); 7.03 (d, 2H); 7.29 (m, 5H); 7.75 (d, 2H); 7.88 (d, 1H). HPLC-MS (Method B): *m/z* = 537 (M+1); R_t = 4.32 min.

<u>Step D: 8-(3-Aminoazepan-1-yl)-7-benzyl-1,3-dimethyl-3,7-dihydropurine-2,6-dione, TFA (6)</u>

N-(1-(7-Benzyl-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-8-yl)azepan-3-yl)-4-methylbenzenesulfonamide (6C) (100 mg, 0.19 mmol) was dissolved in hydrobromic acid (48%, 5 ml) and benzene (0.07 ml), and phenol (61.4 mg, 0.65 mmol) was added. The mixture was heated to reflux for three hours, and after cooling 20 ml of EtOAc was added. The layers were separated, and the aqueous layer washed with 20 ml of EtOAc. pH was adjusted to 11 with 10M sodium hydroxide. The aqueous layer was extracted with diethyl ether (3 x 20 ml), and the combined organic layers were dried over sodium sulphate and the solvent was evaporated. The crude product was dissolved in 5 ml of DCM and 0.5 ml of trifluoroacetic acid was added. The solvents were evaporated and the crude product was purified by prepa-

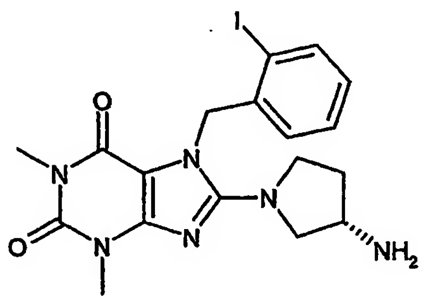
rative HPLC (method A1, Rt = 7.63 min). Evaporation of the solvent gave the title compound as an oil.

Yield: 8 mg (8%).

HPLC-MS (Method B): m/z = 383 (M+1); R_t = 2.00 min.

Example 7

(S) 8-(3-Aminopyrrolidin-1-yl)-7-(2-iodobenzyl)-1,3-dimethyl-3,7-dihydropurine-2,6-dione. HCl



Step A: (S) (1-(7-(2-Iodobenzyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-8-yl)pyrrolidin-3-yl)carbamic acid tert-butyl ester (7A)

8-Chloro-7-(2-iodobenzyl)-1,3-dimethyl-3,7-dihydropurine-2,6-dione (5A) (100 mg, 0.23 mmol) and (3S)-(-)-3-(tert-butoxycarbonylamino)pyrrolidine (216 mg, 1.16 mmol), and triethylamine (0.32 ml, 2.32 mmol) were dissolved in 20 ml of 2-propanol and the mixture was subjected to microwaves (method F, 130°C, 300W) for three hours. The solvents were evaporated and the crude product was purified by preparative HPLC (method A1, Rt = 12.99 min.). Evaporation of the solvent afforded compound (7A) as white crystals.

Yield: 132 mg (98%).

HPLC-MS (Method B): m/z = 581 (M+1); R_t = 4.42 min.

Step B: (S) 8-(3-Aminopyrrolidin-1-yl)-7-(2-iodobenzyl)-1,3-dimethyl-3,7-dihydropurine-2,6-dione. HCl (7)

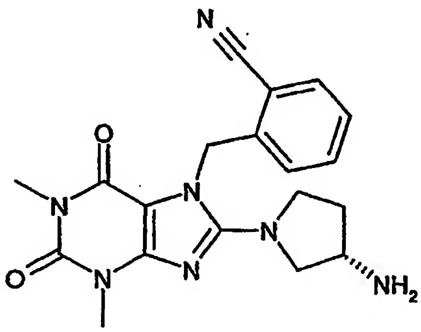
(S) (1-(7-(2-Iodobenzyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-8-yl)pyrrolidin-3-yl)carbamic acid tert-butyl ester (7A) (132 mg, 0.23 mmol) was reacted and purified as described in example **3**, step B, to give the title compound as white crystals.

Yield: 84 mg (72%). Mp. 119 - 223°C.

^{1}H-NMR (MeOD, 300 MHz) δ: 2.03 (m, 1H); 2.34 (m, 1H); 3.26 (s, 3H); 3.52 (m, 4H); 3.65 (m, 2H); 3.90 (m, 2H); 5.45 (d, 1H); 5.52 (d, 1H); 6.73 (d, 1H); 7.04 (m, 1H); 7.32 (m, 1H); 7.92 (d, 1H). HPLC-MS (Method B): *m/z* = 481 (M+1); R_t = 1.89 min.

Example 8

(S) 2-(8-(3-Aminopyrrolidin-1-yl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-7-ylmethyl)benzonitrile. HCl



<u>Step A: (S) (1-(7-(2-Cyanobenzyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-8-yl)pyrrolidin-3-yl)carbamic acid tert-butyl ester (8A)</u>

2-(8-Chloro-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-7-ylmethyl)benzonitrile (1A) (100 mg, 0.30 mmol) was reacted with (3S)-(-)-3-(tert-butoxycarbonylamino)pyrrolidine (282 mg, 1.52 mmol), and purified as described in example 7, <u>step A</u>, to afford compound (8A) as white crystals.

Yield: 117 mg (81%).

Prep. HPLC, (method A1): R_t =11.50 min.

HPLC-MS (Method B): *m/z* = 480 (M+1); R_t = 3.75 min.

<u>Step B: (S) 2-(8-(3-Aminopyrrolidin-1-yl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-7-ylmethyl)benzonitrile. HCl (8)</u>

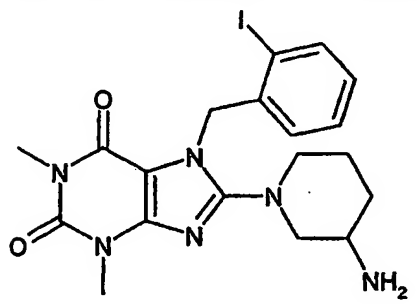
(S) (1-(7-(2-Cyanobenzyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-8-yl)pyrrolidin-3-yl)carbamic acid tert-butyl ester (8A) (117 mg, 0.24 mmol) was reacted and purified as described in example 3, <u>step B</u>, to give the <u>title compound</u> as white crystals.

Yield: 51 mg (50%). Mp.104 - 117°C.

^{1}H-NMR (MeOD, 300 MHz) δ: 2.08 (m, 1H); 2.40 (m, 1H); 3.26 (s, 3H); 3.52 (s, 3H); 3.53 - 3.78 (m, 3H); 3.92 (m, 2H); 5.71 (d, 1H); 5.78 (d, 1H); 7.13 (d, 1H); 7.47 (m, 1H); 7.62 (m, 1H); 7.80 (d, 1H). HPLC-MS (Method B): *m/z* = 380 (M+1); R_t = 1.34 min.

Example 9

8-(3-Aminopiperidin-1-yl)-7-(2-iodobenzyl)-1,3-dimethyl-3,7-dihydropurine-2,6-dione. TFA



8-Chloro-7-(2-iodobenzyl)-1,3-dimethyl-3,7-dihydropurine-2,6-dione (5A) (100 mg, 0.23 mmol) and 3-aminopiperidine dihydrochloride (202 mg, 1.16 mmol) were reacted and purified as described in example **1**, <u>step B,</u> to give the <u>title compound</u> as oily brown crystals.

Yield: 19 mg (13%).

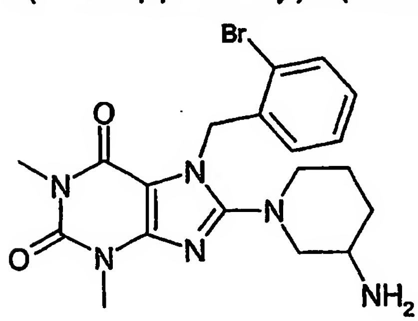
Prep. HPLC (method A1): R_t = 7.70 min.

^{1}H-NMR (MeOD, 300 MHz) δ: 1.62 (m, 2H); 1.74 (m, 1H); 2.08 (m, 1H); 2.94 (m, 1H); 3.18 (m, 2H); 3.28 (s, 3H); 3.46 (m, 1H); 3.54 (s, 3H); 3.70 (m, 1H); 5.35 (s, 2H); 6.78 (d, 1H); 7.04 (m, 1H); 7.32 (m, 1H); 7.92 (d, 1H).

HPLC-MS (Method B): *m/z* = 495 (M+1); R_t = 2.09 min.

Example 10

8-(3-Aminopiperidin-1-yl)-7-(2-bromobenzyl)-1,3-dimethyl-3,7-dihydropurine-2,6-dione. TFA



Step A: 7-(2-Bromobenzyl)-8-chloro-1,3-dimethyl-3,7-dihydropurine-2,6-dione (10A)

8-Chlorotheophylline (10 g, 46.6 mmol) was dissolved in 250 ml of DMF and 8 ml of DIEA, and 2-bromobenzyl bromide (12.2 g, 48.9 mmol) was added. The mixture was stirred at 65°C for 2 hours. The reaction mixture was added 20 ml of EtOAc and 250 ml of cold water. The white precipitate was collected by filtration to afford compound (10A) as white crystals.

Yield: 17.2 g (96%). Mp. 165.4 - 166.7°C.

^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 3.37 (s, 3H); 3.60 (s, 3H); 5.67 (s, 2H); 6.57 (d, 1H); 7.20 (m, 2H); 7.62 (d, 1H). HPLC-MS (Method B): m/z = 385 (M+2); R_t = 3.77 min.

Step B: 8-(3-Aminopiperidin-1-yl)-7-(2-bromobenzyl)-1,3-dimethyl-3,7-dihydropurine-2,6-dione. TFA (10)

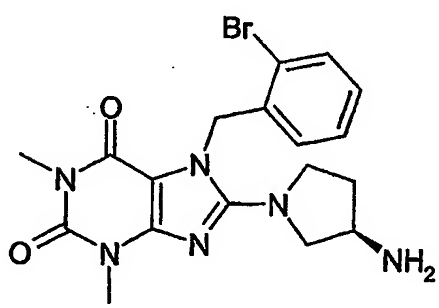
7-(2-Bromobenzyl)-8-chloro-1,3-dimethyl-3,7-dihydropurine-2,6-dione (10A) (100 mg, 0.26 mmol) and 3-aminopiperidine dihydrochloride (226 mg, 1.31 mmol) were dissolved in 2-propanol (20 ml), triethylamine (0.109 ml, 0.78 mmol) and DMF (5 ml) and subjected to microwaves (method F, 130°C, 300W) for ten hours. The solvents were evaporated and the crude product was purified by preparative HPLC, (method A1, Rt = 7.52 min.) to give the title compound as a brown oil.

Yield: 10 mg (7%).

HPLC-MS (Method B): m/z = 447 (M+); R_t = 2.05 min.

Example 11

(R) 8-(3-Aminopyrrolidin-1-yl)-7-(2-bromobenzyl)-1,3-dimethyl-3,7-dihydropurine-2,6-dione. TFA



Step A: (R) (1-(7-(2-Bromobenzyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-8-yl)pyrrolidin-3-yl)carbamic acid tert-butyl ester (11 A)

7-(2-Bromobenzyl)-8-chloro-1,3-dimethyl-3,7-dihydropurine-2,6-dione (10A) (100 mg, 0.26 mmol) and (3R)-(+)-3-(tert-butoxycarbonylamino)pyrrolidine (243 mg, 1.30 mmol) were reacted and purified as described in example 3, step A, to give compound (11A) as brown crystals.

Yield: 44 mg (32%). Mp. 104 - 106°C.

Prep. HPLC, (method A1): Rt = 12.66 min.

^{1}H-NMR (MeOD, 200 MHz) δ: 1.40 (s, 9H); 1.83 (m, 1H); 2.07 (m, 1H); 3.25 (s, 3H); 3.37 (m, 1H); 3.48 - 3.78 (m, 6H); 4.04 (m, 1H); 5.57 (s, 2H); 6.74 (d, 1H); 7.23 (m, 2H); 7.62 (m, 1H).

HPLC-MS (Method B): *m/z* = 535 (M+2); R_t = 4.08 min

Step B: (R) 8-(3-Aminopyrrolidin-1-yl)-7-(2-bromobenzyl)-1,3-dimethyl-3,7-dihydropurine-2,6-dione. TFA (11)

(R) (1-(7-(2-Bromobenzyl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-8-yl)pyrrolidin-3-yl)carbamic acid tert-butyl ester (11A) (44 mg, 0.08 mmol) was dissolved in MeCN (1 ml), water (1 ml), and TFA (0.32 ml), and the mixture was stirred at room temperature for 2 days. The solvents were evaporated and the crude product was purified by preparative HPLC (method A1, Rt = 6.92 min.) to give the title compound as a brown oil.

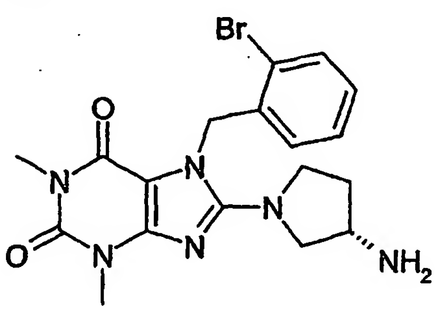
Yield: 13 mg (30%).

^{1}H-NMR (MeOD, 300 MHz) δ: 2.05 (m, 1H); 2.35 (m, 1H); 3.25 (s, 3H); 3.50 - 3.74 (m, 6H); 3.90 (m, 2H); 5.54 (d, 1H); 5.61 (d, 1H); 6.80 (dd, 1H); 7.21 (dt, 1H); 7.30 (dt, 1H); 7.63 (dd, 1H).

HPLC-MS (Method B): *m/z* = 433 (M+1); R_t = 1.83 min.

Example 12

(S) 8-(3-Aminopyrrolidin-1-yl)-7-(2-bromobenzyl)-1,3-dimethyl-3,7-dihydropurine-2,6-dione. HCl



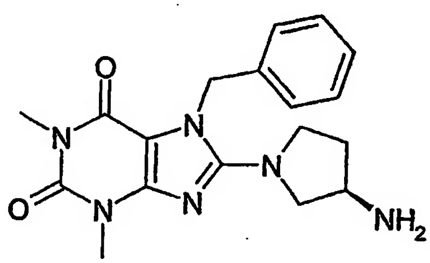
7-(2-Bromobenzyl)-8-chloro-1,3-dimethyl-3,7-dihydropurine-2,6-dione (10A) (100 mg, 0.26 mmol) and (S)-(-)-3-aminopyrrolidine (112 mg, 1.30 mmol) were dissolved in 2-propanol (20 ml) and DMF (5 ml) and subjected to microwaves (method F, 130°C, 300W) for 10 hours. The solvents were evaporated and the crude product was purified by preparative HPLC (method A1, Rt = 6.92 min.) to give the title compound as brown crystals.

Yield: 50 mg (41%). Mp. 215 - 217°C.

^{1}H-NMR (MeOD, 200 MHz) δ: 2.04 (m, 1H); 2.33 (m, 1H); 3.25 (s, 3H); 3.48 - 3.78 (m, 6H); 3.90 (m, 2H); 5.53 (d, 1H); 5.60 (d, 1H); 6.80 (dd, 1H); 7.25 (m, 2H); 7.63 (dd, 1H). HPLC-MS (Method B): m/z = 433 (M+); R_t = 1.80 min.

Example 13

(R) 8-(3-Aminopyrrolidin-1-yl)-7-benzyl-1,3-dimethyl-3,7-dihydropurine-2,6-dione. HCl



7-Benzyl-8-chloro-1,3-dimethyl-3,7-dihydropurine-2,6-dione (2A) (100 mg, 0.33 mmol) and (R)-(+)-3-aminopyrrolidine (141 mg, 1.64 mmol) were reacted and purified as described in example 12 to give the title compound as brown crystals.

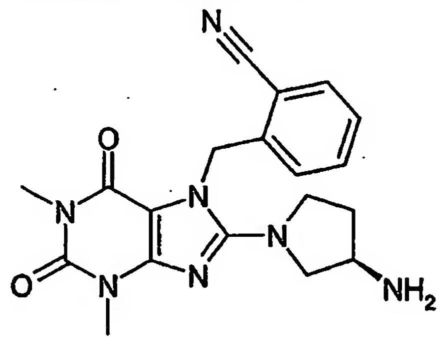
Yield: 73 mg (57%). Mp. 103 - 114°C.

Prep. HPLC, (method A1): Rt = 6.38 min.

^{1}H-NMR (MeOD, 200 MHz) δ: 2.08 (m, 1H); 2.35 (m, 1H); 3.27 (s, 3H); 3.49 (s, 3H); 3.55 - 4.00 (m, 5H); 5.52 (d, 1H); 5.63 (d, 1H); 7.12 (m, 2H); 7.29 (m, 3H). HPLC-MS (Method B): m/z = 355 (M+1); R_t = 1.55 min.

Example 14

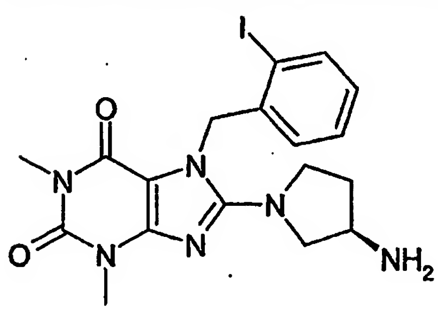
(R) 2-(8-(3-Aminopyrrolidin-1-yl)-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-7-ylmethyl)benzonitrile. HCl



2-(8-Chloro-1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-7-ylmethyl)benzonitrile (1A) (100 mg, 0.30 mmol) and (R)-(+)-3-aminopyrrolidine (131 mg, 1.57 mmol) were reacted and purified as described in example **2**, step B, to give the title compound as brown crystals.
Yield: 125 mg (99%). Mp. 202 - 204°C.
Prep. HPLC, (method A1): Rt = 6.17 min.
^{1}H-NMR (MeOD, 200 MHz) δ: 2.12 (m, 1H); 2.41 (m, 1H); 3.22 (s, 3H); 3.49 (s, 3H); 3.55 - 4.04 (m, 5H); 5.70 (d, 1H); 5.78 (d, 1H); 7.11 (d, 1H); 7.47 (t, 1H); 7.61 (t, 1H); 7.78 (d, 1H).
HPLC-MS (Method B): *m/z* = 380 (M+1); R_t = 1.38 min.

Example 15

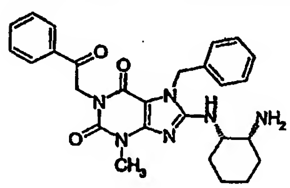
(R) 8-(3-Aminopyrrolidin-1-yl)-7-(2-iodobenzyl)-1,3-dimethyl-3,7-dihydropurine-2,6-dione. HCl



8-Chloro-7-(2-iodobenzyl)-1,3-dimethyl-3,7-dihydropurine-2,6-dione (5A) (100 mg, 0.23 mmol) and (R)-(+)-3-aminopyrrolidine (100 mg, 1.16 mmol) were reacted and purified as described in example **2**, <u>step B</u>, to give the <u>title compound</u> as white crystals.
Yield: 61 mg (51%). Mp. 233 - 235°C.
Prep. HPLC, (method A1): Rt = 7.24 min.
^{1}H-NMR (MeOD, 200 MHz) δ: 2.05 (m, 1H); 2.34 (m, 1H); 3.25 (s, 3H); 3.46 - 3.76 (m, 6H); 3.90 (m, 2H); 5.43 (d, 1H); 5.52 (d, 1H); 6.72 (dd, 1H); 7.03 (dt, 1H); 7.32 (dt, 1H); 7.91 (dd, 1H). HPLC-MS (Method B): *m/z* = 481 (M+1); R_t = 1.88 min.

Example 16 (General procedure (E))

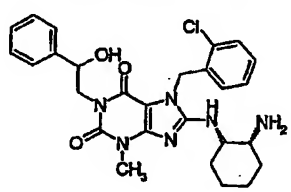
Cis-8-(2-Aminocyclohexylamino)-7-benzyl-3-methyl-1-(2-oxo-2-phenylethyl)-3,7-dihydropurine-2,6-dione



^{1}H NMR (DMSO-d_6): δ 8.10 - 8.01 (m, 2H); 7.82 (s br, 3H); 7.71 (t, 1H); 7.57 (t, 2H); 7.38 - 7.17 (m, 5H); 6.73 (d, 1H); 5.51 - 5.23 (m, 4H); 4.29 - 4.17 (m, 1H); 3.59 (s br, 1H); 3.42 (s, 3H); 1.89 - 1.29 (m, 8H). HPLC-MS (Method Anyone): *m/z* = 487 (M+1); R_t = 3.087 min

Example 17 (General procedure (E))

Cis-8-(2-Aminocyclohexylamino)-7-(2-chlorobenzyl)-1-(2-hydroxy-2-phenylethyl)-3-methyl-3,7-dihydropurine-2,6-dione



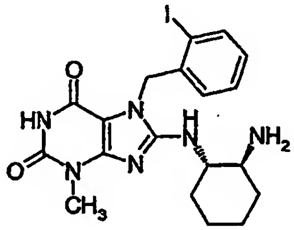
<u>Step B:</u>

Styrene oxide was employed instead of R^5-X

^{1}H NMR (DMSO-d_6): δ 7.79 (s br, 3H); 7.55 - 7,48 (m, 1H); 7,38 - 7,15 (m, 7H); 6,81 - 6,71 (m, 1H); 6,63 - 6,54 (m, 1H); 5.59 - 5.35 (m, 2H); 4.93 - 4.81 (m, 1H); 4.24 (s br, 1H); 4.14 - 4.04 (m, 1H); 3.41 (s, 3H); 1.86 - 1.29 (m, 8H). HPLC-MS (Method Anyone): *m/z* = 523 (M+1); R_t = 3.058 min.

Example 18 (General procedure (G))

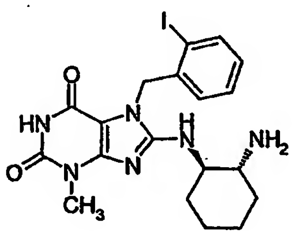
Trans-8-(2-(S)-Amino-cyclohexyl-(S)-amino)-7-(2-iodo-benzyl)-3-methyl-3,7-dihydro-purine-2,6-dione



^{1}H NMR (DMSO-d_6): δ 10.68 (s, 1H); 9.92 (d, 1H); 7.85 (s br, 3H); 7.32 (t, 1H); 7.12 - 6.97 (m, 2H); 6.42 (d, 1H); 5.36 - 4.96 (dd, 2H); 3.86 - 3.68 (m, 1H); 3.36 (s, 3H); 3.09 - 2.93 (m, 1H) 2.08- 1.12 (m, 8H). HPLC-MS (Method Anyone): *m/z* = 495 (M+1); R_t = 2.313 min.

Example 19 (General procedure (G))

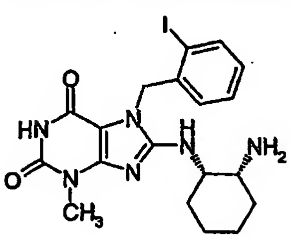
Trans-8-(2-(R)-Amino-cyclohexyl-(R)-amino)-7-(2-iodo-benzyl)-3-methyl-3,7-dihydro-purine-2,6-dione



^{1}H NMR (DMSO-d_6): δ 10.68 (s, 1H); 7.92 (d, 1H); 7.85 (s br, 3H); 7.33 (t, 1H); 7.10-7.00 (m, 2H); 6.42 (m, 1H); 5.29 (d, 1H); 5.03 (d, 1H); 3.77 (m, 1H); 3.36 (s, 3H); 3.01 (m, 1H); 1.98 (m, 2H); 1.69 (m, 2H); 1.42 (m, 1H); 1.24 (m, 3H). HPLC-MS (Method h8): *m/z* = 495 (M+1); R_t = 3.70 min.

Example 20 (General procedure (G))

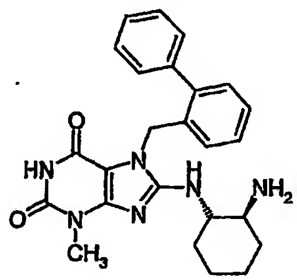
Cis-8-(2-Amino-cyclohexylamino)-7-(2-iodo-benzyl)-3-methyl-3,7-dihydro-purine-2,6-dione



^1H NMR (DMSO-d_6): δ 10.67 (s, 1H); 7.91 (d, 1H); 7.76 (s br, 3H); 7.31 (t, 1H); 7.04 (t, 1H); 6.73 (d, 1H); 6.44 (d, 1H); 5.39 - 5.14 (m, 2H); 1.06 (s br, 1H); 3.59 (s br, 1H); 3.35 (s, 3H); 1.86-1-28 (m, 8H). HPLC-MS (Method Anyone): m/z = 495 (M+1) R_t = 2.313

Example 21 (General procedure (G))

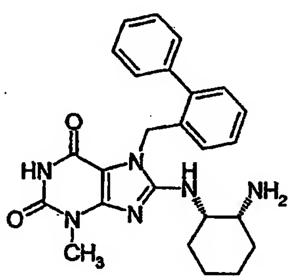
Trans-8-(2-(S)-Amino-cyclohexyl-(S)-amino)-7-biphenyl-2-ylmethyl-3-methyl-3,7-dihydro-purine-2,6-dione



^1H NMR (DMSO-d_6): δ 10.58 (s, 1H); 7.87 (s br, 3H); 7.55-7.23 (m, 7H); 7.03 (d, 1H); 6.58 (d, 1H); 5.37 (d, 1H); 5.11 (d, 1H); 3.78 (m, 1H); 3.34 (s, 3H); 3.02 (m, 1H); 2.03 (m, 2H); 1.74 (m, 2H); 1.45 (m, 1H); 1.26 (m, 3H). HPLC-MS (Method h8): m/z = 445 (M+1); R_t = 4.03 min.

Example 22 (General procedure (G))

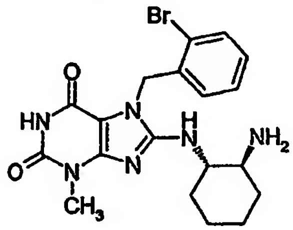
Cis-8-(2-Amino-cyclohexylamino)-7-biphenyl-2-ylmethyl-3-methyl-3,7-dihydro-purine-2,6-dione



^1H NMR (DMSO-d_6): δ 10.57 (s, 1H); 7.79 (s br, 3H); 7.50-7.22 (m, 8H); 6.66 (d, 1H); 6.54 (d, 1H); 5.39 (d, 1H); 5.24 (d, 1H); 4.22 (m, 1H); 3.55 (m, 1H); 3.32 (s, 3H); 1.80-1.30 (m, 8H). HPLC-MS (Method h8): m/z = 445 (M+1); R_t = 3.92.

Example 23 (General procedure (G))

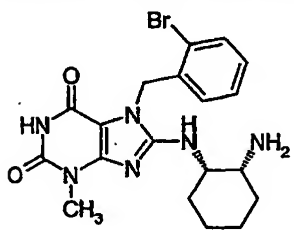
Cis-8-(2-(S)-Amino-cyclohexyl-(S)-amino)-7-(2-bromo-benzyl)-3-methyl-3,7-dihydro-purine-2,6-dione



^{1}H NMR (DMSO-d_6): δ 10.68 (s, 1H); 7.87 (s br, 3H); 7.69 (d, 1H); 7.37 - 7.19 (m, 2H); 7.045 (d, 1H); 6.51 (d, 1H); 5.46 - 5.08 (dd, 2H); 3.87 - 3.71 (m, 1H); 3.36 (s, 3H); 3.10 - 2.92 (m, 1H); 2.09 - 1.09 (m, 8H). HPLC-MS (Method Anyone): m/z = 449 (M+1); R_t = 1.932 min.

Example 24 (General procedure (G))

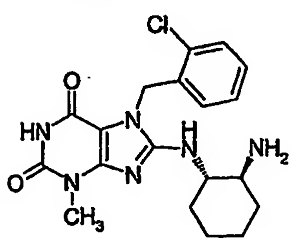
Cis-8-(2-Amino-cyclohexylamino)-7-(2-bromo-benzyl)-3-methyl-3,7-dihydro-purine-2,6-dione



^{1}H NMR (DMSO-d_6): δ 10.67 (s, 1H); 7.77 (s br, 3H); 7.67 (d, 1H); 7.36 - 7.17 (m, 2H); 6.74 (d, 1H); 5.51 - 5.26 (dd, 2H); 4.22 (s br, 1H); 3.58 (s br, 1H); 3.35 (s,3H); 1.87 - 1.28 (m, 8H). HPLC-MS (Method Anyone): m/z = 449 (M+1); R_t = 1.926

Example 25 (General procedure (G))

Trans-8-(2-(S)-Amino-cyclohexyl-(S)-amino)-7-(2-chloro-benzyl)-3-methyl-3,7-dihydro-purine-2,6-dione

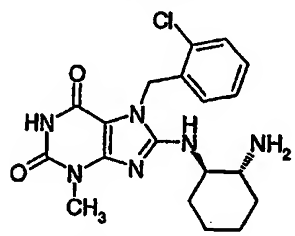


^{1}H NMR (DMSO-d_6): δ 10.68 (s br, 1H); 7.86 (s br, 3H); 7.56 - 7.48 (m, 1H); 7.37- 7.22 (m, 2H); 7.10 - 6.99 (m, 1H); 6.61 - 6.52 (m, 1H) 1.51 - 5.15 (dd, 2H); 3.86 - 3.69 (m. 1H); 3.36

(s, 3H); 3.08 - 2.93 (m, 1H); 2.09 - 1.12 (m, 8H). HPLC-MS (Method Anyone): *m/z* = 403 (M+1); R_t 2.184 min.

Example 26 (General procedure (G))

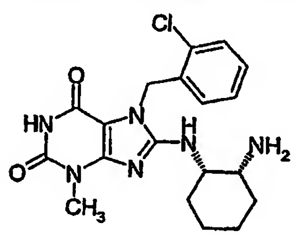
Trans-8-(2-(R)-Amino-cyclohexyl-(R)-amino)-7-(2-chloro-benzyl)-3-methyl-3,7-dihydro-purine-2,6-dione



^{1}H NMR (DMSO-d_6): δ 10.68 (s, 1H); 7.92 (s br, 3H); 7.52 (d, 1H); 7.30 (t+t, 2H); 7.08 (d, 1H); 6.57 (d, 1H); 5.44 (d, 1H); 5.21 (d, 1H); 3.77 (m, 1H); 3.36 (s, 3H); 3.02 (m, 1H); 2.00 (m, 2H); 1.68 (m, 2H); 1.42 (m, 1H); 1.23 (m, 3H).

Example 27 (General procedure (G))

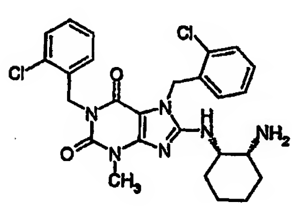
Cis-8-(2-Amino-cyclohexylamino)-7-(2-chloro-benzyl)-3-methyl-3,7-dihydro-purine-2,6-dione



^{1}H NMR (DMSO-d_6): δ 10.68 (s br, 1H); 7.75 (s br, 3H); 7.505 (dd, 1H); 7.35 - 7.22 (m, 2H); 7.76 - 6.58 (m, 2H); 5.52 - 5.33 (dd, 2H); 4.22 (s br, 1H); 3.58 (s, 1H); 3.14 (s, 3H); 1.87 - 1.27 (m, 8H). HPLC-MS (Method Anyone): *m/z* = 403 (M+1); R_t = 2.192 min.

Example 28 (General procedure (E))

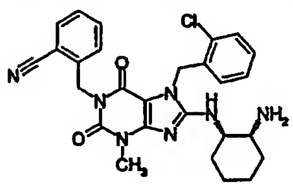
Cis-8-(2-Amino-cyclohexylamino)-1,7-bis-(2-chloro-benzyl)-3-methyl-3,7-dihydro-purine-2,6-dione



^{1}H NMR (DMSO-d_6): δ 7.79 (s br, 3H); 7.50-7.37 (m, 2H); 7.35-7.10 (m, 4H); 6.86 (d, 1H); 6.77 (d, 1H); 5.58 (d, 1H); 5.46 (dd, 2H); 4.99 (s, 2H); 4.27 (m, 1H); 3.60 (m, 1H); 3.46 (s, 3H); 1.80-1.30 (m, 8H). (Method h8): *m/z* = 527 (M+1); R_t = 5.12 min.

Example 29 (General procedure (E))

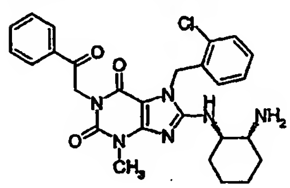
Cis-2-[8-(2-Amino-cyclohexylamino)-7-(2-chloro-benzyl)-3-methyl-2,6-dioxo-2,3,6,7-tetrahydro-purin-1-ylmethyl]-benzonitrile



^{1}H NMR (DMSO-d_6): δ 7.80 (s + d, 4H); 7.57 (t, 1H); 7.50 (d, 1H); 7.41 (t, 1H); 7.29 (t +t, 2H); 7.09 (d, 1H); 6.86 (d, 1H); 6.68 (d, 1H); 5.48 (dd, 2H); 5.12 (s, 2H); 4.26 (m, 1H); 3.60 (m, 1H); 3.44 (s, 3H); 1.80-1.35 (m, 8H). (Method h8): *m/z* = 518 (M+1); R_t = 4.72 min.

Example 30 (General procedure (E))

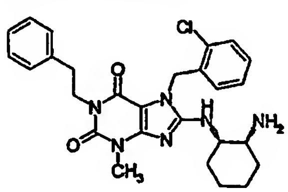
Cis-8-(2-Amino-cyclohexylamino)-7-(2-chloro-benzyl)-3-methyl-1-(2-oxo-2-phenyl-ethyl)-3,7-dihydro-purine-2,6-dione



^{1}H NMR (DMSO-d_6): δ 8.01 (d, 2H); 7.77 (s br, 3H); 7.69 (t, 1H); 7.55 (t, 2H); 7.49 (d, 1H); 7.29 m, 2H); 6.86 (d, 1H); 6.69 (d, 1H); 5.46 (dd, 2H); 5.25 (dd, 2H): 4.28 (m, 1H); 3.64 (m, 1H); 3.46 (s, 3H); 1.80-1.30 (m, 8H). (Method h8): *m/z* = 521 (M+1); R_t = 4.85 min.

Example 31 (General procedure (E))

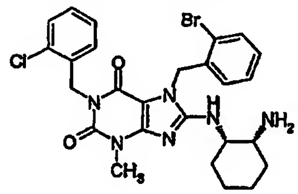
Cis-8-(2-Amino-cyclohexylamino)-7-(2-chloro-benzyl)-3-methyl-1-phenethyl-3,7-dihydro-purine-2,6-dione



^{1}H NMR (DMSO-d_6): δ7.78 (s br, 3H); 1.52 (d, 1H); 7.35-7.24 (m, 4H); 7.24-7.12 (m, 3H); 6.79 (d, 1H); 6.61 (d, 1H); 5.47 (dd, 2H); 4.24 (m, 1H); 3.94 (t, 2H); 3.59 (m, 1H); 3.43 (s, 3H); 2.73 (t 1H); 1.80-1.30 (m, 8H). (Method h8): *m/z* = 507 (M+1); R_t = 5.10 min.

Example 32 (General procedure (E))

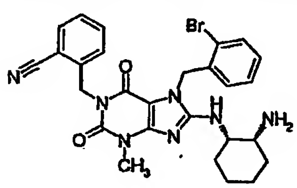
Cis-8-(2-Amino-cyclohexylamino)-7-(2-bromo-benzyl)-1-(2-chloro-benzyl)-3-methyl-3,7-dihydro-purine-2,6-dione



^{1}H NMR (DMSO-d_6): δ7.76 (s br, 3H); 7.66 (d, 1H); 7.42 (d, 1H); 7.40-7.15 (m, 4H); 6.87 (d, 1H); 6.77 (d, 1H); 6.62 (d, 1H); 5.41 (dd, 2H); 4.98 (s, 2H); 4.27 (m, 1H); 3.61 (m, 1H); 3.46 (s, 3H); 1.80-1.35 (m, 8H). (Method h8): *m/z* = 573 (M+1); R_t = 5.37 min

Example 33 (General procedure (E))

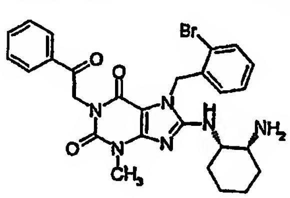
Cis-2-[8-(2-Amino-cyclohexylamino)-7-(2-bromo-benzyl)-3-methyl-2,6-dioxo-2,3,6,7-tetrahydro-purin-1-ylmethyl]-benzonitrile



^{1}H NMR (DMSO-d_6): δ7.78 (d, 1H); 7.74 (s br, 3H); 7.67 (d, 1H); 7.57 (t, 1H); 7.41 (t, 1H); 7.31 (t, 1H); 7.22 (t, 1H); 7.09 (d, 1H); 6.86 (d, 1H); 6.61 (d, 1H); 5.42 (dd, 2H); 5.11 (s, 2H); 4.26 (m, 1H); 3.61 (m, 1H); 3.45 (s, 3H); 1.80-1.35 (m, 8H). (Method h8): *m/z* = 562 (M+1); R_t = 4.88

Example 34 (General procedure (E))

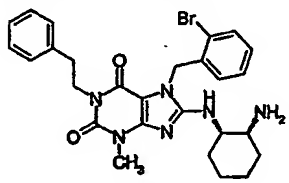
Cis-8-(2-Amino-cyclohexylamino)-7-(2-bromo-benzyl)-3-methyl-1-(2-oxo-2-phenyl-ethyl)-3,7-dihydro-purine-2,6-dione



^{1}H NMR (DMSO-d_6): δ 8.01 (d, 2H); 7.74 (s br, 3H); 7.67 (m, 2H); 7.55 (m, 2H); 7.32 (t, 1H); 7.25 (t, 1H); 6.88 (d, 1H); 6.61 (d, 1H); 5.41 (dd, 2H); 5.25 (dd, 2H); 4.28 (m, 1H); 3.63 (m, 1H); 3.46 (s, 3H); 1.80-1.35 (m, 8H). (Method h8): m/z = 567 (M+1); R_t = 5.02 min.

Example 35 (General procedure (E))

Cis-8-(2-Amino-cyclohexylamino)-7-(2-bromo-benzyl)-3-methyl-1-phenethyl-3,7-dihydro-purine-2,6-dione



^{1}H NMR (DMSO-d_6): δ 7.75 (s br, 3H); 7.69 (d, 1H); 7.35-7.10 (m, 7H); 6.80 (d, 1H); 6.54 (d, 1H); 5.43 (dd, 2H); 4.23 (m, 1H); 3.94 (t, 2H); 3.61 (m, 1H); 3.43 (s, 3H); 2.73 (2H); 1.80-1.30 (m, 8H). (Method h8): m/z = 551 (M+1); R_t = 5.28 min.

IN VIVO TESTING OF COMBINATION TREATMENT

Methods

Non-fasted anaesthetised pigs (n = 5) were given valine-pyrrolidide (250 μmol/kg; V) to inhibit DPP IV activity throughout the experiment. An intraveneous (iv) infusion of GLP-1 (0.75 pmol/kg/min) was started, during which an iv glucose load (0.2 g/kg over 9 min) was given. 90 min after the end of the first GLP-1 infusion, candoxatril was administered iv (5 mg/kg; C), and the protocol (GLP-1 and glucose infusion) was repeated. In addition, during both GLP-1 infusions, simultaneous blood samples were taken from the carotid artery, the renal, femoral, hepatic and portal veins for determination of arterio-venous GLP-1 concentration differences. Samples were analysed for blood glucose, insulin, glucagon and GLP-1 concentrations.

Results

Compared to V alone, co-administration of C significantly increased the plasma levels (plateau level, 73 ± 13 vs 122 ± 22 pmol/l; $P < 0.018$; Fig 1) and the plasma $t_{1/2}$ (2.5 ± 0.2 vs 8.3 ± 0.9 min; $P < 0.002$) of GLP-1 The metabolic clearance rate of GLP-1 during DPP IV inibition alone (25.1 ± 3.9 ml/kg/min) was significantly ($P < 0.022$) reduced during combined DPP IV and NEP 24.11 inhibition (11.7 ± 1.2 ml/kg/min). Calculation of the individual organ extractions revealed that renal clearance was reduced ($P < 0.047$) by combined DPP IV and NEP 24.11 inhibition (44.1 ± 5.4% compared to 58.7 ± 4.3% for DPP IV inhibition alone). Extraction across the extremities, portal bed or liver was not affected by dual DPP IV/ NEP 24.11 inhibition compared to DPP IV inhibition alone. Combined VP+C treatment significantly ($P < 0.016$) reduced the glucose excursion ($\Delta AUC_{27\text{-}67\,min}$, 28 ± 10 mmol/l x min; Fig 2) compared to VP alone ($\Delta AUC_{27\text{-}67\,min}$, 59 ± 4 mmol/l x min), and the glucose elimination rate was increased (11.6 ± 1.3 vs 6.6 ± 0.5 %/min, VP+C vs VP alone, $P < 0.016$). VP+C significantly ($P < 0.008$) potentiated insulin secretion ($AUC_{27\text{-}67\,min}$, 3606 ± 668 vs 6486 ± 1064 pmol/l x min, VP vs VP+C; Fig 3). Interestingly, glucagon concentrations were also elevated after dual inhibition of DPP IV and NEP24.11 compared to DPP IV inhibition alone, increasing from 9 ± 2 to 18 ± 2 pmol/l ($P < 0.045$; Fig 4) in the 20 min period following C administration (before the start of the GLP-1 infusion). The overall glucagon excursion was also greater ($P < 0.030$) with dual DPP IV/NEP 24.11 inhibition ($AUC_{0\text{-}107\,min}$, 3007 ± 775 mmol/l x min) compared to DPP IV inhibition alone ($AUC_{0\text{-}107\,min}$, 585 ± 185 mmol/l x min; Fig 4).

n = 5

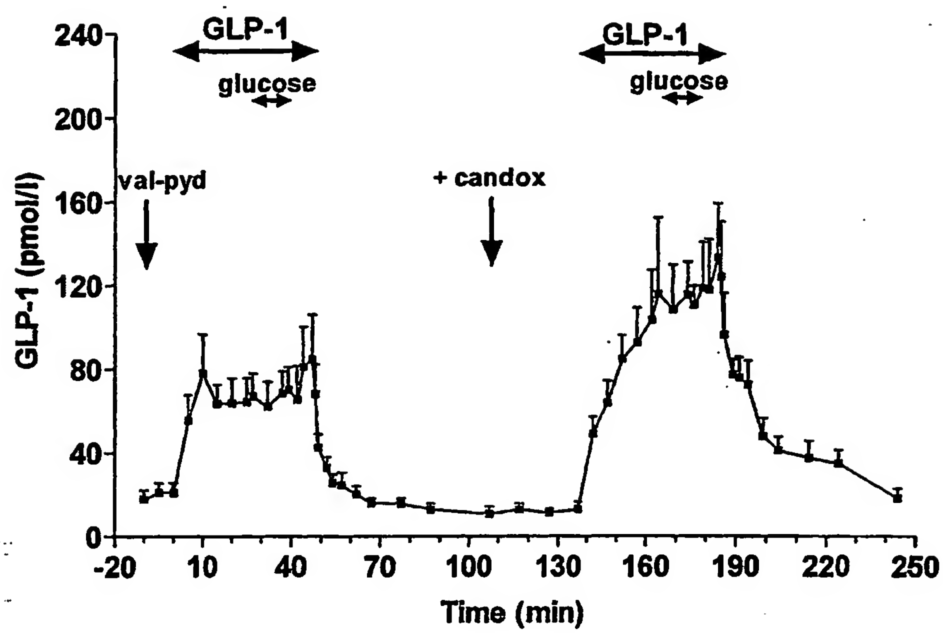


Figure 1 Effect of valine pyrrolidide with and without co.administration of candoxatril on GLP-1 and glucose-mediated total GLP-1 levels

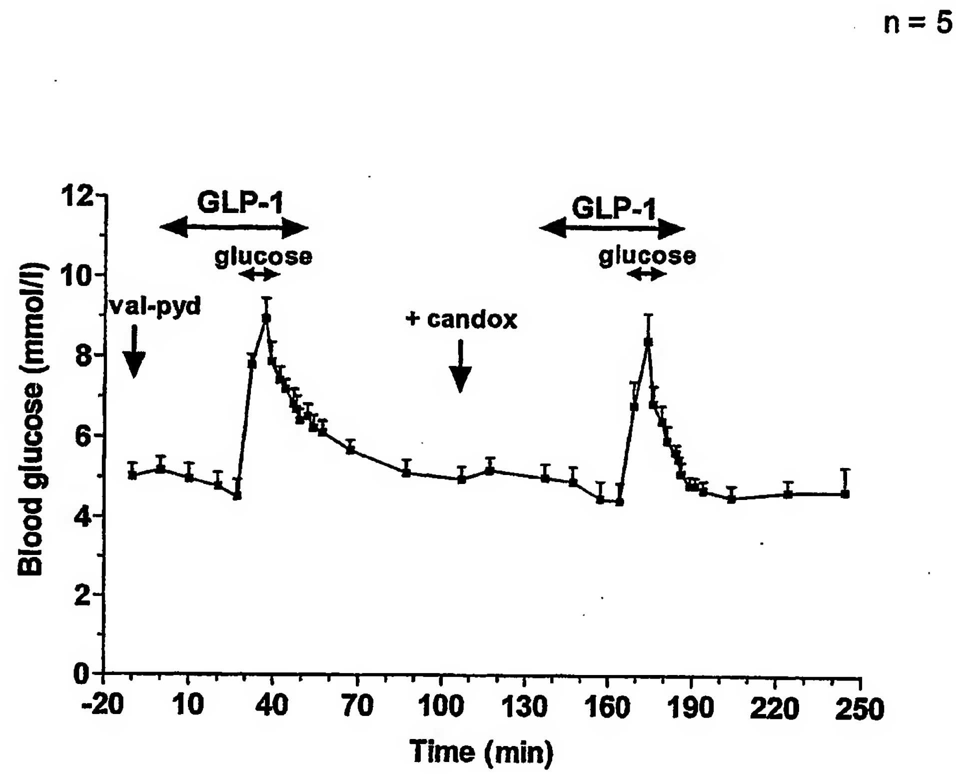


Figure 2 Effect of valine pyrrolidide with and without co-administration of candoxatril on GLP-1 -mediated glucose profiles

n = 5

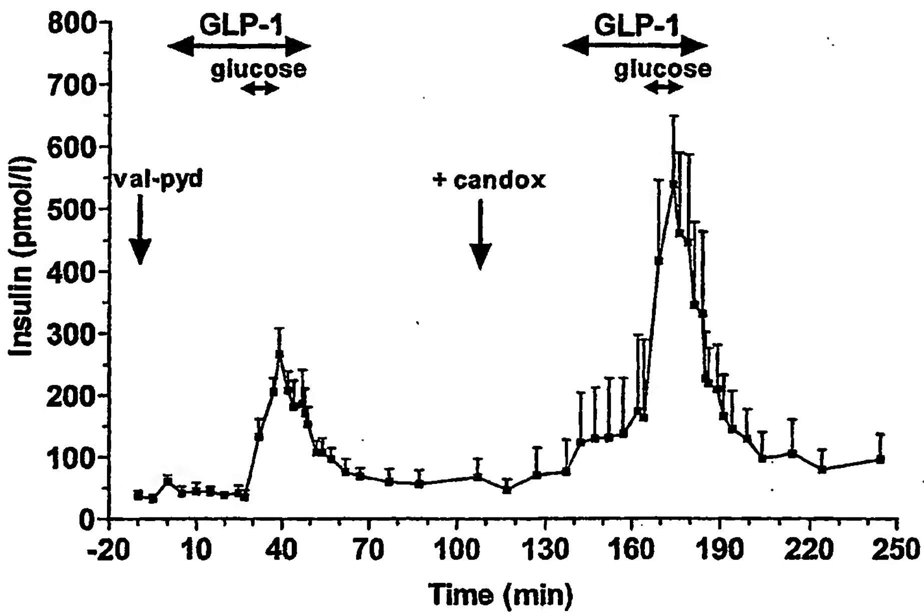


Figure 3 Effect of valine pyrrolidide with and without co-administration of candoxatril on GLP-1 and glucose-mediated insulin profiles

n = 4

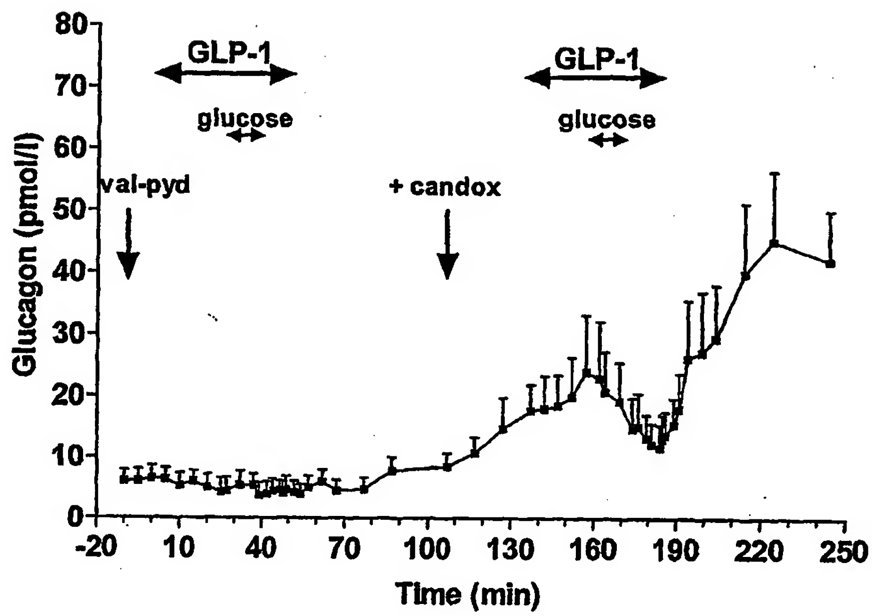


Figure 4 Effect of valine pyrrolidide with and without co.administration of candoxatril on GLP-1 and glucose-mediated glucagon profiles

PHARMACOLOGICAL METHODS

Methods for measuring the activity of compounds which inhibit the enzymatic activity of CD26/DPP-IV

Summary.

Chemical compounds are tested for their ability to inhibit the enzyme activity of purified CD26/DPP-IV. Briefly, the activity of CD26/DPP-IV is measured *in vitro* by its ability to cleave the synthetic substrate Gly-Pro-p-nitroanilide (Gly-Pro-pNA). Cleavage of Gly-Pro-pNA by DPP-IV liberates the product p-nitroanilide (pNA), whose rate of appearance is directly proportional to the enzyme activity. Inhibition of the enzyme activity by specific enzyme inhibitors slows down the generation of pNA. Stronger interaction between an inhibitor and the enzyme results in a slower rate of generation of pNA. Thus, the degree of inhibition of the rate of accumulation of pNA is a direct measure of the strength of enzyme inhibition. The accumulation of pNA is measured spectrophotometrically. The inhibition constant, Ki, for each compound is determined by incubating fixed amounts of enzyme with several different concentrations of inhibitor and substrate.

Materials:

The following reagents and cells are commercially available:

Porcine CD26/DPP-IV (Sigma D-7052), Gly-Pro-pNA (Sigma G0513).

Assay buffer: 50 mM Tris pH 7.4, 150 mM NaCl, 0,1% Triton X-100.

Gly-Pro-pNA cleavage-assay for CD26:

The activity of purified CD26/DPP-IV is assayed in reactions containing:

70 µl assay buffer

10 µl inhibitor or buffer

10 µl substrate (Gly-Pro-pNA from a 0.1M stock solution in water) or buffer

10 µl enzyme or buffer

Reactions containing identical amounts of enzyme, but varying concentrations of inhibitor and substrate, or buffer as control, are set up in parallel in individual wells of a 96-well ELISA plate. The plate is incubated at 25 °C and absorbance is read at 405 nm after 60 min incubation. The inhibitor constants are calculated by non-linear regression hyperbolic fit and the result is expressed as inhibition constant (Ki) in nM.

<u>Diabetes model</u>

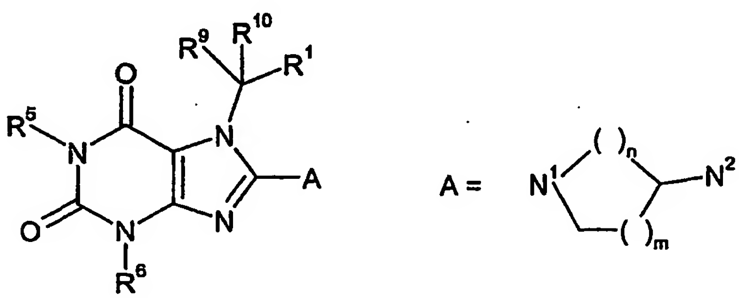
The Zucker Diabetic Fatty (ZDF) rat model can be used to investigate the effects of the compounds of the invention on both the treatment and prevention of diabetes as rats of this sub-strain are initially pre-diabetic although develop severe type 2 diabetes characterised by increased HbA1c levels over a period of 6 weeks. The same strain can be used to predict the clinical efficacy of other anti-diabetic drug types. For example, the model predicts the potency and limited clinical efficacy of thiazolidinedione insulin sensitizers compounds.

CLAIMS

1. A pharmaceutical preparation comprising a combination of a Dipeptidyl Peptidase IV inhibitor and a Neutral Endopeptidase inhibitor, or a pharmaceutically acceptable salt of either or both of these.

2. A pharmaceutical preparation according to claim 1 wherein the Dipeptidyl Peptidase IV is a N-substituted adamantyl-amino-acetyl-2-cyano pyrrolidine or a N-(substituted glycyl)-4-cyano pyrrolidine.

3. A pharmaceutical preparation according to claim 1 wherein the Dipeptidyl Peptidase IV is a compound of formula I



Formula I

wherein A may be attached at either N1 or at N2 to the purine system and each n and m is one or two independently

R1 is aryl optionally substituted with one or more R2 independently or heteroaryl optionally substituted with one or more R2 independently;

R2 is H; C1-C7 alkyl; C2-C7 alkenyl; C2-C7 alkynyl; C3-C7 cycloalkyl; C3-C7 cycloheteroalkyl; -NHCOR3; -NHSO2R3; -SR3; -SOR3; -SO2R3; -OCOR3; -CO2R4; -CON(R4)2; -CSN(R4)2; -NHCON(R4)2; -NHCSN(R4)2; -NHCONNH2; -SO2N(R4)2; -OR4; cyano; nitro; halogen, wherein each alkyl, alkenyl, alkynyl, cycloalkyl and cycloheteroalkyl is optionally substituted with one or more R3 independently;

R3 is C1-C10 alkyl; C2-C10 alkenyl; C2-C10 alkynyl; C3-C7 cycloalkyl; aryl; heteroaryl; OR11; N(R11)2; SR11, wherein each alkyl, alkenyl, alkynyl, cycloalkyl, aryl and heteroaryl is substituted with one or more R11 independently;

R4 is H; C1-C10 alkyl; C2-C10 alkenyl; C2-C10 alkynyl; C3-C7 cycloalkyl; C3-C7 cycloheteroalkyl; aryl; aryl-C1-C5 alkyl; heteroaryl; heteroaryl-C1-C5 alkyl, wherein each alkyl, al-

kenyl, alkynyl, cycloalkyl, cycloheteroalkyl, aryl, aryl-C1-C5 alkyl, heteroaryl, and heteroaryl-C1-C5 alkyl is substituted with one or more R11 independently;

R5 is H; C1-C10 alkyl; C2-C10 alkenyl; C2-C10 alkynyl; C3-C7 cycloalkyl; C3-C7 cycloalkyl-C1-C5 alkyl; C3-C7 cycloheteroalkyl; C3-C7 cycloheteroalkyl-C1-C5 alkyl; aryl; heteroaryl; aryl-C1-C5 alkyl; heteroaryl-C1-C5 alkyl; -OR7; -[(CH2)o-O]p-alkyl, wherein o and p are 1-3 independently, and wherein each alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkyl-C1-C5 alkyl, cycloheteroalkyl, C3-C7 cycloheteroalkyl-C1-C5 alkyl, aryl, aryl-C1-C5 alkyl, heteroaryl, aryl-C1-C5 alkyl, and heteroaryl-C1-C5 alkyl is optionally substituted with one or more R7 independently;

R6 is C1-C10 alkyl; C2-C10 alkenyl; C2-C10 alkynyl; C3-C7 cycloalkyl; C3-C7 cycloheteroalkyl; aryl; heteroaryl; aryl-C1-C5 alkyl; heteroaryl-C1-C5 alkyl; C3-C7 cycloheteroalkyl-C1-C5 alkyl, wherein each alkyl, alkenyl, alkynyl, cycloalkyl, cycloheteroalkyl, C3-C7 cycloheteroalkyl-C1-C5 alkyl, aryl, aryl-C1-C5 alkyl, heteroaryl, and heteroaryl-C1-C5 alkyl is optionally substituted with one or more R11 independently;

R7 is H; =O; C1-C10 alkyl; C2-C10 alkenyl; C2-C10 alkynyl; C3-C7 cycloalkyl; C3-C7 cycloheteroalkyl; aryl; heteroaryl, OR11; N(R11)2; SR11, wherein each alkyl, alkenyl, alkynyl, cycloalkyl, cycloheteroalkyl, aryl, and heteroaryl is optionally substituted with one or more R11 independently;

R8 is C1-C10 alkyl; C2-C10 alkenyl; C2-C10 alkynyl; C3-C7 cycloalkyl; C3-C7 cycloheteroalkyl; aryl; heteroaryl, OR11; N(R11)2; SR11, wherein each alkyl, alkenyl, alkynyl, cycloalkyl, cycloheteroalkyl, aryl, and heteroaryl is optionally substituted with one or more R11 independently;

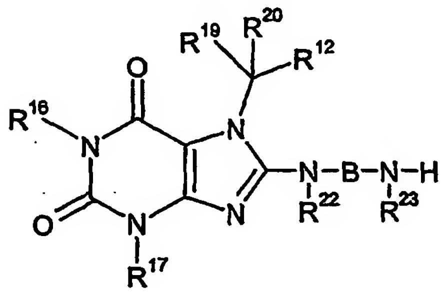
R9 and R10 is independently H, C1-C10 alkyl optionally substituted with one or more R8 independently, halogen;

R11 is H; -CF3; -CCl3; -OCF3; -OMe; cyano; halogen; -OH, COMe; -CONH2; CONHMe; CONMe2; -NO2;

If R9 and R10 is C1-C10 alkyl they may be connected to form a cyclopropyl ring;

if two R4 or two R11 are attached to the same nitrogen they may be connected to form a 3- to 7-membered ring;
or any tautomer, enantiomer, diastereomer or mixture thereof, as well as a salt thereof with a pharmaceutically acceptable acid or base.

4. A pharmaceutical preparation according to claim 1 wherein the Dipeptidyl Peptidase IV is a compound of formula II



Formula II

wherein

B is C2-C6 alkylene; C2-C10 alkenylene; C3-C7 cycloalkylene; C3-C7 cycloheteroalkylene; arylene; heteroarylene; C1-C2 alkylene-arylene; arylene-C1-C2 alkylene; C1-C2 alkylene-arylene-C1-C2 alkylene, wherein each alkylene, alkenylene, cycloalkylene, cycloheteroalkylene, arylene, or heteroarylene is optionally substituted with one or more R14 independently;

R12 is aryl optionally substituted with one or more R13 independently or heteroaryl optionally substituted with one or more R13 independently;

R13 is H; C1-C7 alkyl; C2-C7 alkenyl; C2-C7 alkynyl; C3-C7 cycloalkyl; C3-C7 cycloheteroalkyl; -NHCOR14; -NHSO2R14; -SR14; -SOR14; -SO2R14; -OCOR14; -CO2R15; -CON(R15)2; -CSN(R15)2; -NHCON(R15)2; -NHCSN(R15)2; -NHCONNH2; -SO2N(R15)2; -OR15; cyano; nitro; halogen, wherein each alkyl, alkenyl, alkynyl, cycloalkyl and cycloheteroalkyl is optionally substituted with one or more R14 independently;

R14 is C1-C10 alkyl; C2-C10 alkenyl; C2-C10 alkynyl; C3-C7 cycloalkyl; aryl; heteroaryl; OR21; N(R21)2; SR21, wherein each alkyl, alkenyl, alkynyl, cycloalkyl, aryl and heteroaryl is optionally substituted with one or more R21 independently;

R15 is H; C1-C10 alkyl; C2-C10 alkenyl; C2-C10 alkynyl; C3-C7 cycloalkyl; C3-C7 cycloheteroalkyl; aryl; aryl-C1-C5 alkylene; heteroaryl; heteroaryl-C1-C5 alkylene, wherein each alkyl, alkenyl, alkynyl, cycloalkyl, cycloheteroalkyl, aryl, aryl-C1-C5 alkylene, heteroaryl, and heteroaryl-C1-C5 alkylene is optionally substituted with one or more R21 independently;

R16 is H; C1-C10 alkyl; C2-C10 alkenyl; C2-C10 alkynyl; C3-C7 cycloalkyl; C3-C7 cycloheteroalkyl; aryl; heteroaryl; -OR18; -[(CH2)o-O]p-C1-C5 alkyl, wherein o and p are 1-3 independently, and wherein each alkyl, alkenyl, alkynyl, cycloalkyl, cycloheteroalkyl, aryl, and heteroaryl is optionally substituted with one or more R18 independently;

R17 is H; C1-C10 alkyl; C2-C10 alkenyl; C2-C10 alkynyl; C3-C7 cycloalkyl; C3-C7 cycloheteroalkyl; aryl; heteroaryl; aryl-C1-C5 alkylene; heteroaryl-C1-C5 alkylene; C3-C7 cycloheteroalkyl-C1-C5 alkylene, wherein each alkyl, alkenyl, alkynyl, cycloalkyl, cycloheteroalkyl, C3-C7 cycloheteroalkyl-C1-C5 alkylene, aryl, aryl-C1-C5 alkylene, heteroaryl, aryl-C1-C5 alkylene, and heteroaryl-C1-C5 alkylene is optionally substituted with one or more R21 independently;

R18 is H; =O; C1-C10 alkyl; C2-C10 alkenyl; C2-C10 alkynyl; C3-C7 cycloalkyl; C3-C7 cycloheteroalkyl; aryl; heteroaryl, OR21; N(R21)2; SR21; cyano; hydroxy; halogen; -CF3; -CCl3; -OCF3; or -OCH3 wherein each alkyl, alkenyl, alkynyl, cycloalkyl, cycloheteroalkyl, aryl, and heteroaryl is optionally substituted with one or more R21 independently;

R19 is H; C1-C10 alkyl; C2-C10 alkenyl; C2-C10 alkynyl; C3-C7 cycloalkyl; C3-C7 cycloheteroalkyl; aryl; heteroaryl, OR21; N(R21)2; SR21, wherein each alkyl, alkenyl, alkynyl, cycloalkyl, cycloheteroalkyl, aryl, and heteroaryl is optionally substituted with one or more R21 independently;

R20 is H; C1-C10 alkyl optionally substituted with one or more R19 independently; or halogen;

R21 is H; -CF3; -CCl3; -OCF3; -OCH3; cyano; halogen; -OH, -COCH3; -CONH2; -CONHCH3; -CON(CH3)2; -NO2; -SO2NH2; or -SO2N(CH3)2;

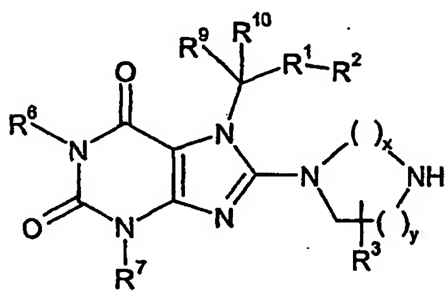
If two R15 or two R21 are attached to the same nitrogen they may be connected to form a 3- to 7-membered ring;

R22 is H; C1-C6 alkyl optionally substituted with one or more R14 independently;

R23 is H; C1-C6 alkyl optionally substituted with one or more R14 independently; or if B is C3-C7 cycloalkylene or C3-C7 cycloheteroalkylene R23 may be a valence bond between the nitrogen to which R23 is attached and one of the atoms in the cycloalkylene or cycloheteroalkylene;

or any tautomer, enantiomer, diastereomer or mixture thereof, as well as a salt thereof with a pharmaceutically acceptable acid or base.

5. A pharmaceutical preparation according to claim 1 wherein the Dipeptidyl Peptidase IV is a compound of formula III



Formula III

wherein

x and y are one or two independently

R1 is C=O; C=S; C1-C2 alkyl optionally substituted with one or more R4 independently; C2 alkenyl substituted with one or more R4 independently; C2 alkynyl; C3-C7 cycloalkyl optionally substituted with one or more R4 independently; C3-C7 cycloheteroalkyl optionally substituted with one or more R4 independently; aryl optionally substituted with one or more R4 independently; aryl C1-C3 alkyl optionally substituted with one or more R4 independently; heteroaryl optionally substituted with one or more R4 independently; heteroaryl C1-C3 alkyl optionally substituted with one or more R4 independently; perhalo C1-C10 alkyl; perhalo C1-C10 alkyloxy;

R2 is H; C1-C7 alkyl optionally substituted with one or more R4 independently; C2-C7 alkenyl optionally substituted with one or more R4 independently; C2-C7 alkynyl optionally

substituted with one or more R4 independently; C3-C7 cycloalkyl optionally substituted with one or more R4 independently; C3-C7 cycloheteroalkyl optionally substituted with one or more R4 independently; aryl optionally substituted with one or more R4 independently; aryl C1-C3 alkyl optionally substituted with one or more R4 independently; heteroaryl C1-C3 alkyl optionally substituted with one or more R4 independently; heteroaryl optionally substituted with one or more R4 independently, -SH; -SR5; SOR5; SO2R5; -CHO; -CH(OR5)2; carboxy; -CO2R4; NHCONNH2; -NHCSNH2; -NHCONH2; -NHCOR4; -NHSO2R5; -O-CO-(C1-C5) alkyl optionally substituted with one or more R4 independently; cyano; nitro; halogen; hydroxy; perhalo C1-C7 alkyl; perhalo C1-C7 alkyloxy; -SO2NH2; -SO2NH(R5); -SO2(R5)2; -CONH2; -CSNH2; -CON2H3; -CONH(R5); -CON(R5)2; C1-C10 alkyloxy optionally substituted with R4 independently; C2-C10 alkenyloxy optionally substituted with R4; C2-C10 alkynyloxy optionally substituted with R4 independently, aryloxy optionally substituted with R4 independently; heteroaryloxy optionally substituted with R4 independently;

R3 is H; C1-C10 alkyl optionally substituted with one or more R4 independently; C2-C10 alkenyl optionally substituted with one or more R4 independently; C2-C10 alkynyl optionally substituted with one or more R4 independently; C3-C7 cycloalkyl optionally substituted with one or more R4 independently; C3-C7 cycloheteroalkyl optionally substituted with one or more R4 independently; aryl optionally substituted with one or more R4 independently; aryl C1-C3 alkyl optionally substituted with one or more R4 independently; heteroaryl C1-C3 alkyl optionally substituted with one or more R4 independently; heteroaryl optionally substituted with one or more R4 independently; C1-C10 alkyl-NH(CH2)1-4NH-aryl optionally substituted with one or more R4 independently; C1-C10 alkyl-NH(CH2)1-4NH-heteroaryl optionally substituted with one or more R4 independently; C1-C10 alkyl-O(CH2)1-4NH-aryl optionally substituted with one or more R4 independently; C1-C10 alkyl-O(CH2)1-4NH-heteroaryl optionally substituted with one or more R4 independently; C1-C10 alkyl-O(CH2)1-4O-aryl optionally substituted with one or more R4 independently; C1-C10 alkyl-O(CH2)1-4O-heteroaryl optionally substituted with one or more R4 independently; C1-C10 alkyl-S(CH2)1-4NH-aryl optionally substituted with one or more R4 independently; C1-C10 alkyl-S(CH2)1-4NH-heteroaryl optionally substituted with one or more R4 independently; C1-C10 alkyl-S(CH2)1-4S-aryl optionally substituted with one or more R4 independently; C1-C10 alkyl-S(CH2)1-4S-heteroaryl optionally substituted with one or more R4 independently; C1-C10 alkyl-O-C1-C5alkyl optionally substituted with one or more R4; -NHCOR4; -NHSO2R5; -O-CO-(C1-C5) alkyl optionally substituted with one or more R4 independently; -SH; -SR5; -SOR5; -SO2R5; -CHO; -CH(OR5)2; carboxy; cyano; nitro; halogen; hydroxy; -SO2NH2; -SO2NH(R5); -

SO2N(R5)2; -CONH2; -CONH(R5); -CON(R5)2; -CSNH2; -CONHNH2; -CO2R4; -NHCNHNH2; -NHCSNH2; -NHCONH2;

R4 is C1-C10 alkyl optionally substituted with one or more R8 independently; C2-C10 alkenyl optionally substituted with one or more R8 independently; C2-C10 alkynyl optionally substituted with one or more R8 independently; C3-C7 cycloalkyl optionally substituted with one or more R8 independently; C3-C7 cycloheteroalkyl optionally substituted with one or more R8 independently; aryl optionally substituted with one or more R8 independently; heteroaryl optionally substituted with one or more R8 independently; amino; amino substituted with one or more C1-C10 alkyl optionally substituted with one or more R8; amino substituted with one or two aryl optionally substituted with one or more R8 independently; heteroaryl optionally substituted with one or more R8 independently; =O; =S; -CO-R5; -COOR5; -O-CO-(C1-C5) alkyl optionally substituted with one or more R8 independently; NH(CH2)1-4NH-aryl; NH(CH2)1-4NH-heteroaryl; -NHCOR5; -SOR5; SO2R5; carboxy; cyano; N-hydroxyimino; nitro; halogen; hydroxy; perhalo C1-C10 alkyl; perhalo C1-C10 alkyloxy; -SH; -SR5; -SO3H; -SO3R5; -SO2R5; -SO2NH2; -SO2NH(R5); -SO2N(R5)2; -CONH2; -CONH(R5); -CON(R5)2; C1-C10 alkyloxy optionally substituted with one or more R8 independently; C2-C10 alkenyloxy optionally substituted with one or more R8 independently; C2-C10 alkynyloxy optionally substituted with one or more R8 independently; aryloxy optionally substituted with one or more R8 independently; heteroaryloxy optionally substituted with one or more R8 independently; and two R4 attached to the same carbon atom may form a spiroheterocyclic system, preferably hydantoin; thiohydantoin; oxazolidine-2,5-dione;

R5 is C1-C10 alkyl optionally substituted with one or more R8 independently; C2-C10 alkenyl optionally substituted with one or more R8 independently; C2-C10 alkynyl optionally substituted with one or more R8 independently; C3-C7 cycloalkyl optionally substituted with one or more R8 independently; C3-C7 cycloheteroalkyl optionally substituted with one or more R8 independently; aryl optionally substituted with one or more R8 independently; aryl C1-C5 alkyl optionally substituted with one or more R8 independently; heteroaryl optionally substituted with one or more R8 independently; heteroaryl C1-C5 alkyl optionally substituted with one or more R8 independently;

R6 is H; C1-C10 alkyl optionally substituted with one or more R4 independently; C2-C10 alkenyl optionally substituted with one or more R4 independently; C2-C10 alkynyl optionally substituted with one or more R4 independently; C3-C7 cycloalkyl optionally substituted with

one or more R4 independently; C3-C7 cycloheteroalkyl optionally substituted with one or more R4 independently; aryl optionally substituted with one or more R4 independently; heteroaryl optionally substituted with one or more R4 independently;

R7 is H; C1-C10 alkyl optionally substituted with one or more R4 independently; C2-C10 alkenyl optionally substituted with one or more R4 independently; C2-C10 alkynyl optionally substituted with one or more R4 independently; C3-C7 cycloalkyl optionally substituted with one or more R4 independently; C3-C7 cycloheteroalkyl optionally substituted with one or more R4 independently; aryl optionally substituted with one or more R4 independently; heteroaryl optionally substituted with one or more R4 independently;

R8 is H, amidoxime; nitro, tetrazole; pentafluorophenyl; -CH2OH; -CHO; -C(OCH3)2; -COCH3; -CF3; -CCl3; -OCF3; -OCH3; -CN; -CO2H; -CO2CH3; -CONH2; -CSNH2; -CON2H3; -SO3H; -SO2NH2; -SO2NHCH3; -SO2N(CH3)2; -SO2 (1-piperazinyl);-SO2 (4-methylpiperazin-1-yl); -SO2 (pyrrolidin-1-yl); -SO2 (piperidin-1-yl); -SO2 (morpholin-4-yl); N-hydroxyimino; -NH2; -NHCH3; -N(CH3)2; -NHCNHNH2; -NHCNHNHCH3; -NHCSNH2; -NHCSNHCH3; -NHCONH2; -NHCONHCH3; -NHCOCH3; -NHSO2CH3; piperazinyl; morhpolin-4-yl; thiomorpholin-4-yl; pyrrolidin-1-yl; piperidin-1-yl; halogen; -OH; -SH; -SCH3; -aminoacetyl; -OPO3H; -OPO2OCH3; -PO3H2; -PO(OCH3)2; PO(OH)(OCH3);

R9 is H; halogen; C1-C10 alkyl optionally substituted with one or more R4 independently

R10 is H; halogen;

or, R9 and R10 may be connected to form a cyclopropyl ring;

or any tautomer, enantiomer, diastereomer or mixture thereof, as well as a salt thereof with a pharmaceutically acceptable acid or base;

with the exception of the following compounds:

1,3-dimethyl-7-(2-oxo-propyl) -8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,

1,3,1',3',7'-pentamethyl-8-piperazin-1-yl-3,7,3',7'-tetrahydro-7,8'-methanediyl-bis-purine-2,6-dione,

3,4,5-trimethoxy-benzoic acid 2-(1,3-dimethyl-2,6-dioxo-8-piperazin-1-yl-1,2,3,6-tetrahydro-purin-7-yl) -ethyl ester,

7-[2-Hydroxy-3-(4-methoxy-phenoxy) -propyl]-3-methyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,
7-[2-hydroxy-2-(4-nitro-phenyl) -ethyl]-3-methyl-8-piperazin-1-yl-3,7,8,9-tetrahydro-purine-2,6-dione,
7-Benzyl-3-methyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,
7-(4-Chloro-benzyl) -3-methyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,
7-(2-Chloro-benzyl) -3-methyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,
7-Ethyl-3-methyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,
3-Methyl-8-piperazin-1-yl-1,7-dipropyl-3,7-dihydro-purine-2,6-dione,
3-Methyl-7-(3-methyl-butyl) -8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,
7-Butyl-3-methyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,
3-Methyl-7-(3-phenyl-propyl) -8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,
7-But-2-enyl-3-methyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,
7-(3-Chloro-but-2-enyl) -3-methyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,
7-Heptyl-3-methyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,
3-Methyl-7-(1-phenyl-ethyl) -8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,
3-Methyl-7-(3-methyl-benzyl) -8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione,
3-Methyl-7-propyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione, and
3-Methyl-7-pentyl-8-piperazin-1-yl-3,7-dihydro-purine-2,6-dione

6. A pharmaceutical preparation according to any one of the claims 1 to 5 wherein the NEP inhibitor is candoxatril

7. A pharmaceutical preparation according to any one of the claims 1 to 5 wherein the NEP inhibitor is a dual NEP/ACE inhibitor

8. A pharmaceutical preparation according to any one of the claims 1 to 6 which furthermore comprises a further antidiabetic.

9. A pharmaceutical preparation according to any one of the claims 1 to 8 which furthermore comprises an Angiotensin Converting Enzyme (ACE) inhibitor.

10. A method of treating a condition that may be regulated or normalised via inhibition of Dipeptidyl Peptidase-IV and Neutral Endopeptidase comprising administering to an individual

in need thereof jointly therapeutically effective amounts of a Dipeptidyl Peptidase-IV inhibitor and a Neutral Endopeptidase inhibitor.

11. A method according to claim 10 wherein the condition is type 2 diabetes.

12. A method according to claim 10 wherein the condition is a condition requiring use of diuretic agents.

13. A method according to claim 12 wherein the condition is hypertension, fluid retention, swelling of the ankles, peripheral oedema, fatigue, dyspnoea, pulmonary oedema, emphysema, peripheral vascular disease, atherosclerosis, intermittent claudication, angina pectoris, re-occlusion of coronary arterial grafts, cerebrovascular stroke, ischaemic heart disease, myocardial infarction, valvular heart disease, congenital heart disease, cardiomyopathy, or fluid retentive states.

14. A method according to any one of the claims 10 to 13 wherein the Dipeptidyl Peptidase-IV is a compound according to claim 3.

15. A method according to any one of the claims 10 to 13 wherein the Dipeptidyl Peptidase-IV is a compound according to claim 4.

16. A method according to any one of the claims claims 10 to 13 wherein the Dipeptidyl Peptidase-IV is a compound according to claim 5

17. A method according to any one of the claims 10 to 16 wherein the Neutral Endopeptidase is candoxatril.

18. A method according to any one of the claims 10 to 17 wherein the Dipeptidyl Peptidase-IV inhibitor and a Neutral Endopeptidase inhibitors are administered simultaneously, separately, sequentially or in the form of a dual Dipeptidyl Peptidase-IV/Neutral Endopeptidase inhibitor.